GENETICS

A periodical record of investigations bearing on heredity and variation

Founded in 1916 by GEORGE H. SHULL

with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

VOLUME 44 NOVEMBER, 1959 NO. 6, PART 3

TABLE OF CONTENTS

New Policies for **Genetics** i

SWAMINATHAN, M. S., and B. R. MURTY, Aspects of asynapsis in plants. I. Random and non random chromosome associations 1271

SIMMONDS, N. W., and E. HARRISON, The genetics of reaction to pepper vein-banding virus 1281

DAVIS, ROWLAND, Asexual selection in *Neurospora crassa* 1291

JAMES, ALLEN P., The spectrum of severity of mutant effects. I. Haploid effects in yeast 1309

PARSONS, P. A., Genotypic-environmental interactions for various temperatures in *Drosophila melanogaster* 1325

CLAYBERG, CARL D., Cytogenetic studies of precocious meiotic centromere division in *Lycopersicon esculentum* Mill. 1335

CLARKE, C. A., and P. M. SHEPPARD, The genetics of *Papilio dardanus*, Brown. I. Race *cenea* from South Africa 1347

KRIMBAS, COSTAS B., Comparison of the concealed variability in *Drosophila willistoni* with that in *Drosophila prosaltans* 1359

MORPURGO, G., and G. SERMONTI, Reactivation by manganous chloride of spores inactivated by nitrogen mustard 1371

PUBLISHED MONTHLY AT AUSTIN, TEXAS
BY GENETICS, INC.

(Date of issue, March 15, 1960)

EDITORIAL BOARD

CLARENCE P. OLIVER, *Managing Editor*,
WILSON S. STONE, *Managing Editor*,
University of Texas

GEORGE W. BEADLE
California Institute of Technology

R. ALEXANDER BRINK
University of Wisconsin

WILLIAM E. CASTLE
University of California

JAMES F. CROW
University of Wisconsin

EVERETT R. DEMPSTER
University of California

L. C. DUNN
Columbia University

JOHN W. GOWEN
Iowa State College

A. D. HERSHEY
Carnegie Institution of Washington

DONALD F. JONES
Connecticut Agricultural Experiment Station

M. M. RHOADES
Indiana University

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

CURT STERN
University of California

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Wisconsin

VOLUME 44 NOVEMBER, 1959 NO. 6, PART 3

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $8.00 net a year for complete volumes (January–November). Foreign postage, 50 cents additional. Single copies, $1.50 each, postpaid.

As available, back volumes may be had at $14.00 each and single issues of previous volumes at $2.50 per copy, postpaid. The Business Office will supply information on request as to volumes and numbers available.

Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., Business Office, Experimental Science Bldg. 122, University of Texas, Austin 12, Texas.

Remittances should be made payable to GENETICS, INC.

Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Experimental Science Building 122, University of Texas, Austin 12, Texas. 3579 should be sent to this address also.

Second-class mail privileges authorized at Austin, Texas.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

The publication of the papers in this Number of Genetics was made possible by a National Science Foundation Grant-G7194 which was given in part for support of publication of manuscript backlog. The Editors express appreciation for the support.

NEW POLICIES FOR *GENETICS*

Effective with Volume 45, 1960

1. GENETICS will be changed from a bimonthly to a monthly journal.

2. The annual volume of the journal will include a minimum of 1,000 pages. The Editors expect to include enough additional pages to print all acceptable manuscripts if funds are available.

3. The subscription rate will be $12.00 for a complete volume (January-December). Foreign postage will be $1.00 extra. Single copies will cost $1.50.

4. Contributions to GENETICS may be in the field of genetics proper, or in any related scientific field if the work reported is primarily of interest to geneticists.

An author is expected to submit to the Editors two typewritten copies of a manuscript, including an original typed copy. Manuscripts must be double spaced throughout, including the footnotes, tabular material, legends and Literature Cited.

5. Each paper published will show the date that the manuscript was received at the Editorial Office. Ordinarily, papers will appear in print in the order they were received as manuscripts, but manuscripts will be classified according to whether they are short or long and the two will be processed on different printing schedules.

a) Short papers are those which are no more than four printed pages in length, including tables and figures. They must conform to the standards and to the general usage in GENETICS, and will be reviewed for quality and scientific merit. Every effort will be made to publish short papers at an accelerated rate. Hence they may appear out of order of date of receipt in comparison to long papers. If a paper is more than four printed pages in length, it will be treated as a long paper in the printing schedule.

b) Long papers are those which are longer than four and up to a maximum of 20 printed pages including tables and figures. They will be accepted for printing as in the past. Each monthly issue of the journal will include both long and short papers.

Papers longer than 20 printed pages will not be accepted for printing except by special vote of the Editorial Board. The author will be expected to pay the costs for printing and handling the extra pages. The additional pages will increase the length of the volume rather than replace another paper. An author who submits a long paper can expect a delay in printing of the paper because of the extra time needed for processing by the reviewers, the Board, and the Editors.

6. In order to help us expedite the printing schedule, an author should prepare his manuscript according to the following instructions. Failure to do so can lead to delay of appearance of the paper in print and may result in charges against the author for extra printing costs.

Second Printing 1969 / University of Texas Printing Division, Austin

a) Manuscripts must conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of Literature Cited, and inclusion of a Summary.

b) A gene symbol should be defined or identified with the mutant character the first time the symbol is used in a manuscript.

c) Excessive footnotes are to be avoided. Footnotes to text statements may be included in the text (parenthetically if necessary).

d) Tables must be typewritten on separate pages with double spacing throughout, and must be arranged to conform to journal page size (5 x 7⅜).

A table should include title, column headings, and footnotes in accordance with general usage. The author is expected to send a copy of the tables in form such that the printer can understand how to set the headings and the body of the table. A table that can be fitted only lengthwise on a page and that fills only a part of the page cannot be accepted.

Very complex tables, such as those using headings that cannot be set in type or using many symbols, can be accepted only if the author includes a reproducible photograph which can be reduced to page size.

An author should be guided by reasonableness as to the number of tables and amount of tabular material he submits. He may be requested to delete some of the material if that seems advisable.

Reference tables with a large amount of detailed data will not be printed. However, any extensive tabular material that cannot be printed will, on request, be kept on file at the editorial office, provided two copies are furnished by the author. A footnote to a text table based upon the material in the reference table can be used to refer to the information kept on file.

e) Legends for figures must be typewritten and double spaced on separate pages.

Material for figures should be original drawings. Illustrations should be mounted in final form for engraving. Clear photographs of line drawings usually are satisfactory. Figures should be prepared so that they will be legible after being reduced to a size to fit the printed page. Photographs and drawings substantially larger than a typewritten page are likely to be damaged in the mail and should not be sent to the Editors. Reviewing of the manuscript is facilitated if photographic copies of the figures are included.

f) Drawings, mating-type charts, chemical structural formulas, and other sketches or complex charts made on typewritten pages cannot be accepted unless a photograph acceptable for engraving is included. The illustration which is photographed must be legible and understandable to the reader. It is preferable to treat the illustration or chart as a figure because it may not be feasible to include it on the printed page exactly at the place referred to in the text.

g) Complex mathematical formulas are preferred in the form of reproducible photographs. If a formula is received in typewritten form, the author must assume responsibility for its accuracy, including differentiation between letters and numbers where misinterpretations might be made (such as the typed letter l and the number 1). An author who sends a typewritten formula which is illegible

or in which the arrangement of the symbols or the intention of the author cannot be readily determined, will be asked to pay for the extra printing costs when corrections in galley proof are made.

Data which are used in the text in semitabular form should be prepared as a regular table unless it is permissible to put part of the material at the bottom of one page and the other part at the top of the next page without duplicating column headings.

7. An author will be expected to pay all extra costs for printing and handling of a manuscript which are made necessary by any of the following conditions: changes in galley proof not due to printer's errors; extra pages (more than 20 printed pages) when accepted; changes made necessary after type has been set because the printer could not interpret the intention of the author due to illegibility of the manuscript, inadequately marked or poorly prepared copy of mathematical formulas or symbols, or inadequately organized tables; and preparation of drawings for photographing of charts, chemical formulas and sketches when the author sends copy only on the typewritten pages.

8. The Galton and Mendel Memorial Fund established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to be applied toward the cost of reproducing illustrations and of printing expensive tables and formulae.

9. The manuscript of a published paper will not be returned unless the author so requests.

10. Galley proofs will be sent to authors but page proofs will not be sent. Authors should leave forwarding directions whenever they are away from the address sent with the manuscript or should make other arrangements to have the galleys corrected promptly. Send corrected galley proofs to the Editors.

11. Reprints are to be ordered directly from the Editors at the time galley proof is returned to the Editors. The author will be billed in accordance with a schedule of charges which represents the costs for printing and handling. Copies of the schedule will be available from the Editors.

12. Manuscripts and correspondence should be addressed to the Editors of Genetics, Experimental Science Building 122, University of Texas, Austin 12, Texas.

ASPECTS OF ASYNAPSIS IN PLANTS. I. RANDOM AND NON RANDOM CHROMOSOME ASSOCIATIONS

M. S. SWAMINATHAN AND B. R. MURTY[1]

Indian Agricultural Research Institute, New Delhi, India

Received December 15, 1958

DURING the course of a cytogenetic study of some derivatives of a cross between *Nicotiana tabacum* and *N. rustica*, the population was found to vary in the extent of chromosome synapsis occurring during meiosis in microsporocytes. A detailed cytological study was therefore undertaken in this population and plants showing normal pairing, partial asynapsis and complete asynapsis were isolated. In the progenies of partially asynaptic plants segregation occurred for the extent of synapsis, thereby indicating that the character is genetic. The data from the analysis of chromosome pairing were subjected to Poisson and binomial analyses to see whether any particular pattern of pairing occurs in this allotetraploid material. Such a study revealed some interesting information and hence the Poisson analysis was extended to all reported cases of asynapsis and desynapsis, where detailed data are available. The results are presented in this paper.

MATERIAL AND METHODS

Seeds belonging to the second segregating generation of the sixth backcross of F_1 *N. rustica* × *N. tabacum* to *N. rustica* were obtained through the kind courtesy of DR. HAROLD H. SMITH of the Cornell University. The material belonged to two families, 55379 and 55380, and were grown in the field during 1955–56, along with normal *N. rustica* and *N. tabacum*. F_3 generation was grown during 1956–57. For the study of microsporogenesis, preparations were made by the Propionocarmine schedule (SWAMINATHAN *et al.* 1954). Fixations were carried out from different plants on the same day and in many cases, several fixations were done from the same plant at regular intervals. Estimation of pollen fertility was based on stainability in acetocarmine as well as by functionability as revealed by data on seed setting. For testing for goodness of fit for Poisson or binomial distributions, classes with expected frequencies of less than five were grouped to make up this minimum number, Poisson analysis being applied to plants with low bivalent frequency and binomial analysis for those cases with a proportion 0.1 or more of the normal bivalent frequency.

The two fractions of the binomial distribution are the proportion (p) of the observed bivalent frequency to the potential frequency of bivalents and ($1-p$). Tests for truncated Poisson distribution were made as per the procedure of RAO and CHAKRAVARTHY (1956).

[1] Present address: Dept. of Plant Breeding, Cornell University, Ithaca, N.Y.

RESULTS

Meiosis in asynaptic plants: A rapid examination of pollen fertility in the *rustica-tabacum* population showed that fertility ranged from three to 70 percent. Chromosome associations at diakinesis and metaphase I of meiosis were studied in 23 plants, which showed that the plants with nearly complete pollen sterility were asynaptic (0 to 4 bivalents per cell) and that the plants with 80 to 90 percent pollen sterility were partially asynaptic (5–18 bivalents). The number of plants observed under each category in the population is shown below.

Family	Normal (22–24 II)	Partially asynaptic (5–18 II)	Completely asynaptic	Total
55379	6	1	—	7
55380	11	2	3	16

As the occurrence of univalents at diakinesis and M I could be due to the failure of initial pachytene association (asynapsis) as well as to a precocious terminalization of chiasmata followed by slipping of chiasmata after a normal pachytene pairing (desynapsis), pachytene stages were studied in two plants in which there was no pairing at diakinesis and M I. No pairing occurred at this stage and consequently the plants fall into the asynaptic category. In partially asynaptic plants, however, it was difficult to ascertain precisely the frequency of univalents occurring at pachytene, though several unpaired chromosomes could be observed.

A detailed comparative study was made of meiosis in two plants of each of the three types, i.e., with normal pairing, partial asynapsis and complete asynapsis. All fixations were made on the same day. The data are given in Tables 1, 2 and 3 (see also Figures 1 and 2). There were no detectable morphological differences among these plants. Two bivalents were found to be attached to the nucleolus in the normal synaptic plants. In asynaptic plants, either one bivalent or one to three univalents were found to be associated with the nucleolus. The nucleolus was also comparatively weakly stained in the asynaptic plants. At metaphase I, there was a greater degree of stickiness among chromosomes in normal plants in

TABLE 1

Chromosome associations at diakinesis

Family and plant no.	No. of cells	Mean no. of configurations with Xta/cell				Mean no. of Xta per cell	Remarks
		0	1	2	3		
55379;							
28/1 and 2	45	0.27	1.18	19.17	3.55	50.07	Normal pairing
55380;							
22/1 and 30/1	30	0.61	1.98	19.33	2.32	48.86	Normal pairing
55379; 3/3	20	37.20	4.65	0.75	0	6.15	Partial asynapsis
55380; 5/2	25	46.32	0.84	0	0	0.84	Asynapsis
55380; 6/9	26	46.92	0.39	0.15	0	0.69	Asynapsis
55380; 7/2	15	14.16	11.69	5.23	0	22.15	Partial asynapsis

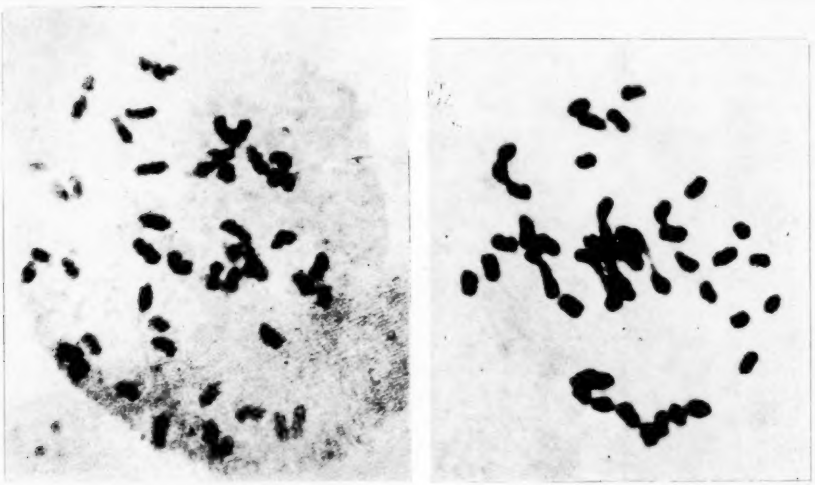
TABLE 2

Frequency of aberrant cells, pollen fertility and seed-setting in normal and asynaptic plants

Plant no.	Percentage of aberrant cells at			Pollen fertility (%)	Seed bearing capsules per plant (%)	No. of seeds per capsule	
	A I	A II	Sporad stage			Selfing	Open pollinated
30/1	21.43	25.42	30.51	70.31	21.54	82.3	90.6
5/2	97.61	69.32	69.31	3.80	6.85	0	71.6
6/9	96.10	94.34	78.18	5.44	7.50	0	36.0
7/2	86.32	78.72	69.93	11.61	17.39	31.5	77.0

comparison with those of the asynaptic ones. Most of the bivalents in asynaptic and partially asynaptic plants were of the rod type with chiasma in only one of the arms. During the subsequent stages of meiosis, there were several irregularities like sticky bridges and lagging chromosomes at A I and A II, unequal distribution of chromosomes at M II and the occurrence of dyads, triads, pentads and micronuclei at the sporad stage. The percentage of cells with such aberrations is given in Table 2. Pollen fertility was very low in the asynaptic plants. There were however some giant, well-stained grains which probably arise as a result of the formation of monads or dyads.

All the plants with complete or partial asynapsis were selfed and also crossed with normal *rustica* pollen. In completely asynaptic plants, no seeds were obtained on selfing. Seed setting was, however, obtained in asynaptic plants both in



1 2

FIGURE 1.—Metaphase I in plant No. 5/2 with 48 univalents.

FIGURE 2.—Metaphase I in plant No. 7/2 with 7 bivalents + 34 univalents.

crosses with normal *rustica* pollen and under open pollinated conditions. Thus, the ovules of these plants were functional. Seed setting was observed in partially asynaptic plants on selfing, in crosses with normal plants and under open pollinated conditions. The total number of capsules formed under open pollinated conditions in the different plants is given in Table 2. Only six to seven percent of such capsules were, however, found to contain seeds in plants number 5/2 and 6/9.

Inheritance of asynapsis: Out of the 23 plants studied during 1955–56, seven plants were either partially or wholly asynaptic. Selfed seeds from the partially asynaptic plant No. 7/2 as well as open pollinated seeds from the others were sown during November 1956, along with *N. rustica* and progenies of normal plants of the *rustica-tabacum* derivative. All this material was, however, damaged severely by a hailstorm which hit Delhi in March, 1957. Only ten plants survived and flowered. Among them, two partially asynaptic and two completely asynaptic plants were found. Asynapsis in the *rustica-tabacum* hybrids therefore appears to be genetically controlled, though it is not possible at this stage to indicate precisely the number of factors that may be concerned.

Poisson analysis of bivalent frequencies in different cells: So far, no case of asynapsis has been recorded in *N. rustica*. In *N. tabacum*, CLAUSEN and CAMERON (1944) identified a "pale sterile" mutant which was also partially asynaptic. This mutant was used by them to develop the monosomic series of *tabacum*. SMITH (1950) who produced the *rustica-tabacum* hybrids used in the present study and who has critically investigated this material has not reported any asynaptic plant in them. From the data now available, their occurrence could only be explained on the assumption of a spontaneous mutation. In view of the hybrid nature of this material and also since *N. rustica* and *N. tabacum* are both allopolyploids, a Poisson analysis of the frequencies of cells with different numbers of bivalents was carried out to find out whether bivalent formation occurs wholly at random. The data are presented in Table 3 (see also Figure 3). Only data from metaphase

TABLE 3

Frequency of bivalents in asynaptic plants at Metaphase I

Plant no.	Number of cells containing a bivalent frequency of													χ^2
	0	1	2	3	4	5	6	7	8	9	10	11	12	
5/2	29	17	5	5	....	....	....	....	....	....	....	....	....	0.661
	26.45	19.84	7.44	1.86										
6/9	32	4	1	0	1	....	....	....	....	....	....	....	....	2.752
	29.30	7.62		1.08										
3/3	0	2	2	1	8	29	6	5	....	....	....	....	....	69.13*
	5.67		8.75	11.22	10.80	8.32	5.34	5.43						
7/2		2					1	8	8	2	15		5	
		5.85					5.25	5.22	5.61	5.35	8.19		6.32	16.596*

The upper figure is the experimental number of cells with the stated number of bivalents; the lower figure is the number expected on the Poisson distribution.
* Significance at 5% level.

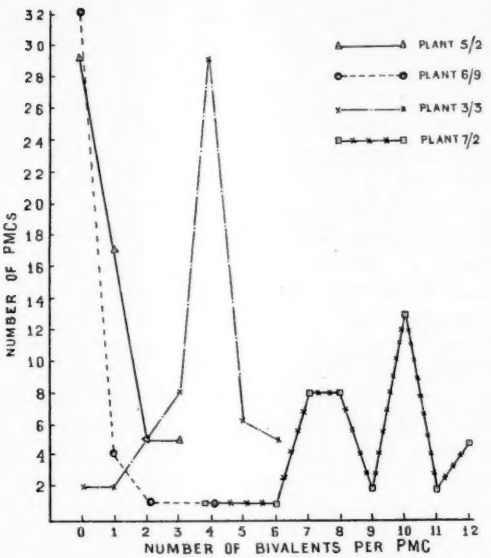


FIGURE 3.—Frequency of occurrence of cells with different bivalent numbers in asynaptic and partially asynaptic plants.

I plates were analyzed, since a large number of cells could be studied at this stage. It can be seen from Table 2 that plants 5/2 and 6/9 showed the lowest χ^2 with a good fit for Poisson distribution. In the other two plants, the deviations from the frequencies expected on the basis of Poisson distributions were significant. Tests for binominal distribution also revealed large deviations from those expected. The curve in the case of plant 7/2, looks bimodal in view of the very low frequency of cells with nine and 11 bivalents. This may, however, be due to the number of cells studied in each class being insufficient. From this data it may be concluded that the bivalents in plants 3/3 and 7/2 are not formed at random.

DISCUSSION

There are two important questions regarding the asynaptic condition in *rustica-tabacum* derivatives which need consideration. First, why is there a gradation in pairing frequency from regular bivalent formation to complete asynapsis? Secondly, what is the deviation from a binomial distribution in the mode of bivalent formation in partially asynaptic plants due to? Regarding the first question, a probable explanation is the existence of a gene dosage effect similar to that observed in Solanum (SWAMINATHAN 1952). Since partially asynaptic plants gave rise to completely asynaptic ones, it is not likely that altogether different genes control the types of asynapsis met with. Lack of chromosome homology cannot be used to explain either partial or complete asynapsis since some plants in the same

population showed regular bivalent formation. Nor can environmental conditions account for the observed differences in pairing since first, the plants were grown under similar conditions and fixations were made on the same day and secondly, the character was inherited. The possibility that asynapsis may result from the pleiotropic effect of the mammoth gene is rendered unlikely by the fact that the *rustica-tabacum* derivatives also segregated at Delhi for the mammoth character (MURTY and SWAMINATHAN 1957). Thus, the asynaptic plants isolated by us and the different degrees of pairing observed in them seem to owe their origin to spontaneous mutation and gene dosage effect respectively.

The nonconformity of bivalent formation in partially asynaptic plants to a Poisson or binomial distribution reveals some interesting possibilities. Certain homologous chromosomes appear to be capable of undergoing synapsis under cellular conditions which are not favorable for the pairing of other chromosomes. Thus, it appears that even within the chromosome complement of a plant, there may be differences among the different chromosomes concerning their requirements for the initiation of pairing. In the genus Nicotiana, spontaneous asynapsis has so far been reported only in *N. tomentosa* (GOODSPEED 1954). In X-rayed progenies of *N. tabacum* and *N. sylvestris*, some asynaptic variants have been recorded. No asynaptic mutants have been reported in *N. rustica*. Whether the bivalents occurring in partially asynaptic *rustica-tabacum* derivatives arise from pairing within a particular genome of *N. rustica* or from pairing within some chromosomes of both the genomes (modified by the presence of some segments of *tabacum* chromosomes) cannot at present be ascertained.

Non randomness in the response of individual chromosomes and even segments of chromosomes with reference to the action of various mutagens and external agencies such as temperature is well-known. REES (1958) presented evidence that in some partially asynaptic plants of Scilla particular chromosomes respond to abnormal conditions within the cells in different ways. A specific chromosome response to temperature was inferred by JAIN (1957) in Lolium from the disproportionally high occurrence of nonsynchronized cells with one or two advanced bivalents. Such specificity may or may not be related to chromosome length. Localization effects have long been studied with references to the distribution of chiasmata. However, no attempt has been made to study the response of homologous pairs of chromosomes to conditions unfavorable for the origin of pairing as in asynapsis, nor to the retention of chiasmata in paired homologues according to the time sequence normal to the species as in desynapsis. In view of the fundamental interest aroused by this problem, the results of different authors, who have reported quantitative data on the chromosome associations found in asynaptic or desynaptic plants, were subjected to a Poisson and binomial analyses. The results are summarized in Tables 4 and 5. It should be mentioned that in all the cases where a significant deviation from Poisson or binomial distribution is reported in Tables 4 and 5, the deviation occurred in all classes and not merely in a single one, i.e., no case of compound or truncated Poisson distribution was found.

From the data in Tables 4 and 5, the following general conclusions can be

TABLE 4

Poisson analysis in desynaptic plants

Material and somatic chromosome number	Plant no.	Mean no. of bivalents per cell	Fit for Poisson and binomial distribution	Remarks
Zea mays (2n=20)*	3695–5	0.30	Very good	Nil
"	3694–5	0.50	Fair	Deviation in first two classes
"	3694–14	0.65	Very good	Nil
"	3693–1	1.34	Fair	Deviation in the first three classes
"	3696–24	2.40	Poor	High deviations in all classes
"	3699–14	4.86	Fair	Sample size small
"	3695–7	6.82	Fair	Sample size small
"	3701–1	8.15	Poor	Deviation in all classes
Pisum sativum (2n=14)†	A_3	3.80	Very poor	Deviation in all classes
"	B_4	3.90	Very poor	Deviation in all classes
"	B_7	3.90	Very poor	Deviation in all classes
"	B_1	4.50	Very poor	Deviation in all classes
Secale cereale (2n=14)‡	100–2	2.38	Fair (P between 0.10 and 0.20)	High Deviation in middle classes
"	100–3	2.63	Very poor	Deviation in all classes
"	1–3	4.40	Very poor	Deviation in all classes
"	6–7	4.98	Very poor	Deviation in all classes
Ulmus glabra (2n=28)§	1	1.05	Very good	Nil
"	7	2.16	Very good	Nil
"	10	2.23	Good	Nil
Matricaria inodora (2n=36)‖	1	5.50	Fair	Sample size only 20 cells
Triticum aestivum (2n=42)¶	1703–1	1.90	Fair	Data pertaining to material
"	1704–1	2.40	Good	Fixed at 10:10 A.M. on 4/8
"	1705–1	2.60	Good	Fixed at 10:10 A.M. on 4/8
"	1695–2	11.50	Very poor	Fixed at 10:10 A.M. on 4/8

* Data from BEADLE (1933).
† Data from KOLLER (1938).
‡ Data from PRAKKEN (1943).
§ Data from EHRENBERG (1949).
‖ Data from VAARAMA (1950).
¶ Data from LI *et al.* (1945).

drawn. (1) In cases of nearly complete asynapsis or desynapsis, an occasional bivalent may be formed at random. (2) As the mean bivalent frequency approached the equivalent of half the potential number of bivalents, a gradual departure from Poisson distribution commences, ending in very wide deviations in those cases where n/2 or more bivalents are formed or where more than the number of bivalents constituting a genome in an alloploid species are formed. (3) The above trend holds good whether the plants concerned are diploids like Rye or *Pisum sativum*, allopolyploids like *Triticum aestivum*, autopolyploid like the Solanum hybrid, or probably secondary polyploids like tomato and maize, or even aneuploids. It thus appears that when conditions conducive to normal chromosome pairing are present only inadequately, there is a definite difference in the ability

TABLE 5

Poisson analysis in asynaptic plants

Material and somatic chromosome number	Plant no.	Mean no. of bivalents per cell	Fit for Poisson and binomial distribution	Remarks
*Triticum aestivum** (2n=42; plants studied had 2n=40)	9 plants	5.77	Very good	Nil
Lycopersicon esculentum† (2n=24)	57–1	4.30	Fair	Deviation in middle classes
"	57–7	5.03	Fair	
"	17–2	5.45	Poor	Deviations in all classes
"	59–4	7.03	Poor	
"	3–3	8.04	Poor	
F_2 *Gossypium hirsutum*‡ × *G. barbadense* (2n=52)	11	6.30	Good	Nil
"	7	6.40	Good	Nil
"	8	6.70	Fair	Sample size small
"	6	7.00	Fair	High deviation in one class
"	1	8.80	Good	Deviations in middle classes
"	3	11.60	Very poor	Deviations in all classes
F_2 and F_3 of amphidiploid§ (*Solanum parodii* × *S. saltense*) 2n=48	358	3.50	Very good	Nil
	190	4.54	Very good	Nil
	362	14.00	Very poor	Deviations in all classes

* Data from Huskins and Hearne (1933).
† Data from Soost (1951).
‡ Data from Beasley and Brown (1942).
§ Data from Swaminathan (1952).

of the different chromosomes of a plant species to behave normally. As a consequence some chromosomes of a complement are unable to undergo synapsis, some others are able to do so, although with a reduced chiasma frequency per bivalent. In allopolyploids, this might be related to the differential requirements of the different genomes with reference to prerequisites for pairing; and in diploids such a situation would suggest that each pair of chromosomes might behave as an independent unit under such conditions. Various theories involving (1) physical changes in the chromosomes or in the cytoplasm, (2) disturbances in the normal time relations between pairing and division of the chromosomes, (3) genetically controlled enzyme deficiencies which exert an influence on the breakage and reunion of chromatids etc., have been proposed to explain the mechanism of asynapsis. Darlington (1957) suggested that such abnormal cells can be considered as containing nuclei where cooperation was restricted over limited distances, while in normal cells the general cooperation within nuclei is permitted by the cytoplasm. However, he did not exclude the responsibility of cooperation between chromosomes in close association. Whatever may be the correct cause, it appears from the present study that genetically controlled threshold effects exist to which homologous chromosomes may respond in a specific and independent manner.

SUMMARY

During a cytogenetic study of the second segregating generation of the sixth backcross of F_1 *N. rustica* × *N. tabacum* to *N. rustica*, segregation was observed for different degrees of asynapsis during microsporogenesis. From a consideration of various alternatives, it is concluded that asynapsis in the population owes its origin to a spontaneous mutation. It is also presumed that gene dosage effects may be responsible for the different degrees of asynapsis found among different plants.

Analysis of the data for Poisson and binomial distribution revealed significant differences on the nature of association of chromosomes between the partially asynaptic and completely asynaptic segregants. Cases reported previously of asynaptic and desynaptic mutants in various plant genera were also subjected to Poisson and binomial analysis. From the results, it is concluded that (a) the odd bivalents found in completely asynaptic plants are formed at random; (b) as the mean bivalent frequency approaches the equivalent of one half the potential number of bivalents, a gradual departure from Poisson or binomial distribution ends in very wide deviations where n/2 or more bivalents are formed, and (c) this type of response wherein specific homologous pairs in a chromosome complement show ability to pair under conditions which lead to asynapsis in other pairs is prevalent among all types of plants, whether they be diploids, secondary polyploids or primary polyploids of various types.

ACKNOWLEDGMENTS

We are very grateful to DR. B. P. PAL, Director, Indian Agricultural Research Institute and DR. S. M. SIKKA, Head of the Division of Botany, for facilities and encouragement. We are also indebted to SHRI B. MARUTI RAM of the Indian Council of Agricultural Research for suggestions and assistance in the Poisson analysis in several examples and to PROF. R. G. D. STEEL of Cornell University for suggestions on the statistical procedures.

LITERATURE CITED

BEADLE, G. W., 1933 Further studies of asynaptic maize. Cytologia **4**: 269–287.

BEASLEY, J. D., and M. S. BROWN, 1942 Asynaptic Gossypium plants and their polyploids. J. Agri. Res. **65**: 421–427.

CLAUSEN, R. E., and D. R. CAMERON, 1944 Inheritance in *Nicotiana tabacum*. XVIII. Monosomic analysis. Genetics **29**: 447–477.

DARLINGTON, C. D., 1957 Messages and movements in the cell. pp. 1–33. *Conference on Chromosomes*. W. E. J. Tjeenk Willink. Zwolle, Netherlands.

EHRENBERG, C. E., 1949 Asynapsis in the elm, *Ulmus glabra*. Hereditas **35**: 1–26.

GOODSPEED, T. H., 1954 *The Genus Nicotiana*. Chronica Botanica Co. Waltham, Mass.

HUSKINS, C. L., and E. M. HEARNE, 1933 Meiosis in asynaptic dwarf oats and wheat. J. Roy. Microscop. Soc. **53**: 109–117.

JAIN, H. K., 1957 Effect of high temperature on meiosis in Lolium. Nucleolar inactivation. Heredity **11**: 23–36.

KOLLER, P. C., 1938 Asynapsis in *Pisum sativum*. J. Genet. **36**: 275–306.

LI, H. W., W. K. PAO, and C. H. LI, 1945 Desynapsis in the common wheat. Am. J. Botany **32**: 92–101.

MURTY, B. R., and M. S. SWAMINATHAN, 1957 Cytogenetic studies in derivatives of *Nicotiana rustica* × *N. tabacum*. Euphytica **6**: 227–236.

PRAKKEN, R., 1943 Studies of asynapsis in rye. Hereditas **29**: 475–495.

RAO, C. R., and I. M. CHAKRAVARTI, 1956 Some small scale sample tests of significance for a Poisson distribution. Biometrics **12**: 264–282.

REES, H., 1958 Differential behaviour of chromosomes in Scilla. Chromosoma **9**: 185–192.

SMITH, H. H., 1950 Differential photoperiodic response from an interspecific gene transfer. J. Heredity **41**: 199–203.

SOOST, R. K., 1951 Comparative cytology and genetics of asynaptic mutants in *Lycopersicon esculentum*. Genetics **36**: 410–434.

SWAMINATHAN, M. S., 1952 Species differentiation and the nature of polyploidy in some species of the genus Solanum, Sect. Tuberarium. Ph.D. thesis. University of Cambridge.

SWAMINATHAN, M. S., M. L. MAGOON, and K. L. MEHRA, 1954 A simple propionocarmine method for plants with small chromosomes. Indian J. Genet. Pl. Breed. **14**: 87–88.

VAARAMA, A., 1950 Cases of asyndesis in *Matricaria inodora* and *Hyoscyamus niger*. Hereditas **36**: 342–362.

THE GENETICS OF REACTION TO PEPPER VEIN-BANDING VIRUS

N. W. SIMMONDS AND E. HARRISON

Regional Research Centre, Imperial College of Tropical Agriculture, Trinidad, W. I., and Agricultural Department, Northern Region, Nigeria

Received March 31, 1959

PEPPER vein-banding virus causes a troublesome disease of cultivated peppers (Capsicum spp.) in Puerto Rico (ADSUAR 1940) and in Trinidad. A rather similar disease occurs in Hawaii (KIKUTA 1947). Serological investigations (PEREZ and ADSUAR 1955) indicate that the virus responsible for the West Indian disease is a strain of potato virus Y. In Trinidad, DALE (1954) found that three distinct seedling reactions were shown by various strains of cultivated pepper and this observation prompted the genetical investigation reported here.

MATERIALS AND METHODS

Four pepper varieties were used:

1. 'Ford Hook' (FH): a commercial sweet pepper from the U.S.A., much grown in Trinidad from imported seed. A strain of *Capsicum annuum* L.
2. 'Puerto Rico Wonder' (PRW): *Capsicum annuum* L., bred in Puerto Rico for resistance to virus and introduced thence to Trinidad by W. T. DALE. ROQUE and ADSUAR (1941) and RIOLLANO, ADSUAR and RODRIGUEZ (1948) have described the breeding of PRW from the susceptible 'California Wonder' crossed by the resistant Mexican pepper 'Cuaresmeno'.
3. 'Large Bell Hot' (LBH): a commercial hot pepper from the U.S.A. introduced to Trinidad by W. T. DALE. A strain of *Capsicum annuum*.
4. 'Hot Yellow' (HY): a shrubby pepper cultivated in Trinidad, acquired from DR. F. J. SIMMONDS. An extremely hardy and long-lived type that bears a large crop of subglobular, orange-yellow, hot fruits. Probably a strain of *Capsicum sinense* Jacquin. The name, 'Hot Yellow', was invented by the writers for the variety, which was received unnamed.

Tests of virus reaction were made on seedling plants in the greenhouse unless otherwise stated. Inoculation was done with the juice of infected 'Ford Hook' plants which was rubbed onto the leaves with the fingers; carborundum powder (grade 400) was used. Seedlings were inoculated first at the 2–4 leaf stage and survivors were reinoculated after about two weeks; sometimes a second reinoculation was carried out about two weeks after the first.

Pollinations were mostly made on plants in pots growing in full sunlight because plants in the greenhouse, though they made good growth, flowered and set poorly. Emasculation was performed just before the buds opened and flowers were protected with pinned pieces of fine muslin or muslin bags which were supported on stakes in rainy weather. Most crosses were about 20 percent successful and

showed no marked reciprocal differences in this respect; an apparent exception to this was LBH × HY in which 20 crosses failed with HY as female parent but in which LBH yielded seeds at the first attempt. This agrees with the results of SMITH (personal communication) who found that the cross *C. annuum* × *C. sinense* could only readily be made with *C. annuum* as the female parent.

RESULTS

Symptoms (cf. DALE *1954)*

Four seedling reactions were distinguished, thus:

1. Necrotic (N): veinal necrosis and leaf abscission; plants commonly rather drooping and flaccid; infection easy and symptoms early (5–6 days); the plant is swiftly reduced to a bare stalk with necrotic streaks; lethal.
2. Necrotic-mosaic (NM): some veinal necrosis and incomplete leaf abscission; surviving leaves show necrotic flecks and severe vein-banding (mosaic); infection difficult and symptoms slower (8–9 days); sublethal, but plants infected as seedlings rarely set fruit. The necrotic symptom is sometimes much delayed (15–20 days).
3. Severe mosaic (SM): severe vein-banding, visible first in youngest leaves, accompanied by some wrinkling, distortion and reduction of size; infection easy, symptoms appearing in 8–9 days; nonlethal but growth very poor.
4. Mild mosaic (MM): mild vein-banding, so mild as to be difficult to recognize in some instances; little or no wrinkling and distortion; infection difficult, symptoms slow (12 or more days); growth little affected. Plants in which no symptoms could be induced were perforce placed in this category, though some of them (see below) were probably of NM genotype.

Reactions of parents and F_1 hybrids

In the seedling stage, parents and F_1 hybrids reacted as follows: showing a necrotic (N) reaction—LBH, LBH × FH, LBH × PRW, LBH × HY; showing a severe mosaic (SM) reaction—FH, FH × PRW; showing a mild mosaic reaction—PRW, HY. The necrosis shown by LBH × HY F_1 was milder than that shown by LBH itself, suggesting the occurrence of incomplete dominance. Although all HY seedlings tested reacted with a mild mosaic, genetical results described below show that the necrotic mosaic phenotype also occurs in this strain. Evidently, genes controlling the necrotic reaction are dominant to all others; the NM reaction was not recognized in the parents of F_1 hybrids (though probably it occurred in the HY strain).

Reactions of healthy mature plants inoculated with the disease were much more difficult to classify and gave clear signs of incomplete dominance thus:

LBH: Vein necrosis, abscission of young leaves and fruits; sublethal.

FH: Severe mosaic with much leaf crinkling and distortion; growth poor.

PRW: Mild (occasionally moderately severe) mosaic; resistant to infection; growth good.

LBH × FH: Variably severe mosaic with leaf curling; some tip die-back; little necrosis; growth poor.

LBH × PRW: Variable mosaic and vein necrosis; much abscission of young leaves and fruits; growth poor.

FH × PRW: Severe mosaic with some leaf crinkling and distortion; growth fair.

Genetical results

Results are summarized in Table 1. They show that two loci are concerned with resistance to disease; the constitutions of plants having the various disease reactions may be symbolized as follows: N—V_1V_2 (e.g. LBH); NM—$V_1v_2v_2$ (e.g. HY. in part); SM—$v_1v_1V_2$ (e.g. FH); MM—$v_1v_1v_2v_2$ (e.g. PRW, HY in part).

The only complication was caused by difficulties of classification of F_2 families of PRW × LBH (line 3 of Table 1). The results for these dihybrid families are given in more detail in Table 2.

The 953 plants tested fell into five groups each of which was raised and tested over a comparatively short period of time (about 4–6 weeks). All the plants within

TABLE 1

Segregations in peppers

Parentage	No. of families	Frequencies N	NM	SM	MM	Ratio	χ^2[n]	P
LBH × FH, F_2	8	209	...	73	...	3:1	0.12[1]	0.7–0.8*
FH × PRW, F_2	5	...	...	105	33	3:1	0.09[1]	0.7–0.8†
LBH × PRW, F_2	15	526	90	232	105	9:3:3:1	94.78[3]	<0.001‡
NM types selfed, F_3	2	...	22	...	9	3:1	0.27[1]	0.5–0.7
(LBH × HY)F_1 × PRW	2	8	16	...	...	1:1	2.67[1]	0.1–0.2
(LBH × HY)F_1 × PRW	1	7	5	5	7	1:1:1:1	0.67[3]	0.7–0.8

* Six families with LBH female, two reciprocal; no heterogeneity.
† Three families with FH female, two reciprocal; no heterogeneity.
‡ See Tables 2 and 3 and analysis in text.

TABLE 2

Dihybrid segregations in peppers.
Groups ranged in descending order of NM class proportions

Group (year)	Frequencies N	NM	SM	MM	Totals	Families	χ^2[n]*
I (1952	41	14	18	10	83	1	....
II (1951)	122	35	46	18	221	5	11.77[12]
III (1958–59)	115	20	55	10	200	5	9.48[12]
IV (1954)	89	11	34	26	160	1	....
V (1952)	159	10	79	41	289	3	1.23[6]
Totals	526	90	232	105	953	.	....

* The values of χ^2 in the last column test heterogeneity between the different families included in one group; none is significant.

any one group were therefore subjected to approximately uniform treatment. Table 2 shows that families within groups were homogeneous with respect to reaction to virus. Between groups, however, treatment was not uniform for there was, perforce, variation in weather, source of inoculum, inoculating operator and observer; it is therefore not surprising to find that the groups are heterogeneous ($\chi^2[12] = 46.02$, $P<0.001$). Four families were tested on two or more occasions and can thus be used as checks on the occurrence of nongenetic variability of classification. Three showed no significant heterogeneity between occasions (though two χ^2 were suggestively large) and one gave clear evidence of heterogeneity. Thus family 342/1 was grown twice in 1952 (see Table 2) with these results (frequencies of N, NM, SM and MM): Group I—41, 14, 18, 10; Group V —50, 3, 25, 11; heterogeneity $\chi^2[3] = 10.72$ and $P = 0.01 - 0.02$.

Taken jointly the five groups diverge significantly from a 9:3:3:1 ratio (χ^2 [3] $= 94.78$, $P<0.001$) but they jointly agree with 9:7, thus: deviation χ^2 [1] $= 0.43$, $P = 0.5 - 0.7$; heterogeneity χ^2 [4] $= 1.58$, $P = 0.8 - 0.9$.

The frequency of the N class is not at fault and this suggests that linkage, if it is involved in the problem, is not tight. The contingency χ^2 appropriate to detect linkage in such data (MATHER 1938) is highly significant (χ^2 [1] $= 36.65$, $P<0.001$). However, calculations, which need not be set out here, show that no reasonable hypothesis based on linkage explains the over-all discrepancy from a 9:3:3:1 ratio. The data of Table 2, however, are heterogeneous and deviations from 9:3:3:1 ratios range from nonsignificant in group I to great in group V. We have seen above that the frequency of the N class accords with expectation and that the discrepancies therefore lie in the other three classes; scrutiny of Table 2 shows that there is a general tendency, increasing down the table, towards deficiency of NM and excess of SM and MM.

All the facts so far adduced tend to show that we are dealing with a 9:3:3:1 ratio sporadically disturbed by environmental influences that cause misclassification of plants other than those of N genotype. The misclassification is twofold: first, NM plants often show very weak and/or late necrotic symptoms and, if none were detected, would have to be classified SM; second, it is known (see below) that v_2v_2 genotypes are much more difficult to infect than genotypes that carry V_2 so that NM plants tend to escape infection, being thus misclassified MM. Obviously, these two errors will jointly tend to produce the observed discrepancy, namely deficiency of NM and excess of SM and MM.

It may be helpful to note here that the obvious method of testing the misclassification hypothesis, namely to raise and test F_3s of plants scored NM, SM and MM in the F_2, is not practicable because NM and SM seedlings either die or are so stunted as to be virtually sterile in later life. Attempts to score whole F_2 families on the basis of F_3 progenies likewise failed because natural virus infection of the F_2 made it impossible to achieve an unbiased sample. Direct proof of the misclassification hypothesis is not therefore available.

The problem may be analyzed as follows. Let p be the probability of misclassifi-

cation of an NM plant as SM and q the probability of failure of infection of an NM plant, then the F_2 will give:

Class	N	NM	SM	MM	Total
Obs.	a	b	c	d	n
Exp.	$\frac{9n}{16}$	$\frac{3n(1-p-q)}{16}$	$\frac{3n(1+p)}{16}$	$\frac{n(1+3q)}{16}$	n

By application of the method of maximum likelihood or, more directly, from the statement

$$\frac{3(1-p-q)}{b} = \frac{3(1+p)}{c} = \frac{(1+3q)}{d}$$

it can be shown that (where $m = b+c+d$)

$$p = \frac{4c-3b-3d}{3m} \text{ and } q = \frac{6d-b-c}{3m}$$

of which the variances and covariance are

$$V_p = \frac{(1+p)\ (4-3p)}{3(b+c+d)} = \frac{49}{9m^3} \cdot c(b+d)$$

$$V_q = \frac{(1+3q)\ (2-q)}{3(b+c+d)} = \frac{49}{9m^3} \cdot d(b+c)$$

$$W_{pq} = -\frac{(1+3q)\ (1+p)}{3(b+c+d)} = -\frac{49}{9m^3} \cdot cd$$

Calculation of the estimates of p and q absorbs two degrees of freedom so that one remains to test goodness of fit. Since p and q depend only on b, c and d, the remaining degree of freedom attaches to the comparison of the frequency of the N class with the other three classes combined—i.e., it applies, in effect, to the test of a 9:7 ratio. Table 3 shows that agreement of calculated with observed values

TABLE 3

Dihybrid segregations in peppers. Estimates of p *and* q *(see text) and calculated segregations (see Table 2)*

Group	p	q	Calculated values N	NM	SM	MM	$\chi^2[n=1]$	P
I	0.0000 ± 0.1782	0.2222 ± 0.1534	46.7	12.1	15.6	8.7	1.58	0.2–0.3
II	0.0842 ± 0.1171	0.0909 ± 0.0905	124.3	34.2	44.9	17.6	0.98	0.3–0.5
III	*0.5098* ± 0.1210	−0.0588 ± 0.0815	112.5	20.6	56.6	10.3	0.13	0.7–0.8
IV	0.1174 ± 0.1383	*0.5211* ± 0.1334	90.0	10.9	33.5	25.6	0.03	0.8–0.9
V	*0.4179* ± 0.0999	*0.4026* ± 0.0951	162.6	9.7	76.8	39.9	0.18	0.5–0.7

(1) Values of p and q written in *italics* differ from zero by more than twice the appropriate standard error.
(2) The values of χ^2 in the penultimate column test agreement of observed frequencies (Table 2) with calculated values given here; each has 3–2 = 1 degree of freedom and all are nonsignificant.

is satisfactory. Values of p and q, it will be noted, vary widely and apparently independently—as would be expected, because there is no obvious reason to expect that failure of NM plants to develop necrotic symptoms should be correlated with failure of infection.

In summary, the dihybrid F_2 data agree with the hypothesis that two independent loci are concerned but that segregation is disturbed in a more or less orderly (or, at least, calculable) fashion by difficulties of classification.

The statement made above that v_2v_2 genotypes are resistant to infection needs justification. Table 4 summarizes information on ease of infection of plants subsequently classified by disease reaction. The data came from the records of inoculation and infection made by one operator over a limited period of time and are not necessarily generally representative but they illustrate the point at issue. Uninfected plants have been excluded on the grounds that they must have included some MM types; and the SM class probably contained a few NM types if the interpretation of aberrant F_2 ratios proposed above is correct. Thus resistance to infection has probably been somewhat overestimated for the NM class. The proportion of this class in the sample of Table 4 that remained uninfected after two inoculations was evidently, therefore, somewhat greater than 12.5 percent; this estimate may be compared with the values of q recorded in Table 3 to which, it will be seen, it is roughly median.

Combining columns 2 and 3 in Table 4, we have: for the V_1v_1 locus, $\chi^2[1] = 0.50$, $P = 0.5$; for the V_2v_2 locus, $\chi^2\ [1] = 99.02$, $P<0.001$; and for the residual interaction, $\chi^2\ [1] = 17.28$, $P<0.001$. The V_2 locus is the most important factor, v_2v_2 genotypes being much more resistant to infection than V_2. Elimination of the errors mentioned in the preceding paragraph would strengthen this conclusion.

Distribution of resistance

The susceptible (N and SM) peppers worked with in this study both belong to *Capsicum annuum*. In a survey of pepper varieties in Puerto Rico, Roque and Adsuar (1941) and Riollano *et al.* (1948) found that 82 out of 84 strains tested were susceptible to the disease; it is not known, however, how many Capsicum species were represented in this collection and, therefore, whether reaction to virus was related to botanical origins.

TABLE 4

Resistance to infection

Reaction	Genotype	Number of plants	Percentage infected at inoculation number		
			1	2	3
N	V_1V_2	261	77.0	19.6	3.4
NM	V_1v_2	24	54.2	33.3	12.5*
SM	v_1V_2	252	84.5	13.9	1.6†
MM	v_1v_2	49	12.2	53.1	34.7*

* Resistance probably slightly underestimated.
† Overestimated (see text).

In a preliminary attempt to define the distribution of resistance in the genus, eight strains representing four Capsicum species were tested with the Trinidad virus. Seeds were supplied by DR. P. G. SMITH as follows (for taxonomic discussion see HEISER and SMITH 1948; SMITH, RICK and HEISER 1951; SMITH and HEISER 1951; and HEISER and SMITH 1953. In the list that follows, strains are distinguished by DR. SMITH's accession numbers; reaction to pepper virus is indicated by "+" for MM reaction in some at least of the four to ten plants tested and by "—" for "no reaction". All eight strains were probably $v_1v_1v_2v_2$. The list is: *C. sinense* Jacq. (1725 from Suriname, +; 1774 from Puerto Rico, —); *C. pubescens* R. and P. (1789 from Peru, +?); *C. frutescens* L. (965 from Costa Rica, +; 1583 from Nicaragua, +); *C. pendulum* Willd. (1244 from Chile, —; 1762 from Peru, —; 1786 from Peru, —).

A note on tobacco mosaic

HOLMES (1934, 1937; and see LUTES 1954) has described genetically controlled variability of reaction to tobacco mosaic in peppers. It was clearly of some interest to determine whether either one of the loci recognized in the course of the present study was the same as the *L* locus of HOLMES. Evidence that different loci were involved in resistance to the two diseases was obtained by inoculating LBH, FH and PRW with tobacco mosaic virus; all seedlings responded with an ill-defined mosaic (presumably the "systemic chlorosis" of HOLMES) and were therefore presumably all *ll*. In view of the facts that tobacco mosaic and pepper virus are known to be quite different viruses and that genes conferring resistance to one virus are not effective against others (HOLMES 1958), this finding is perhaps not very surprising.

CONCLUSIONS

The activities of the two loci in response to pepper vein-banding virus may be stated as follows:

V_1 causes vein necrosis and leaf abscission; also mosaic in the surviving leaves. It is neutral (or nearly so) with respect to resistance to infection. Dominance (judged by results of infection of mature plants and difficulties of F_2 classification) is incomplete.

V_2 causes mosaic, enhances the necrotic-abscission action of V_1 and increases susceptibility to infection. Dominance is virtually complete.

From the plant breeding viewpoint the $v_1v_1v_2v_2$ system offers an extremely useful resistance to the disease, combining, as it does, resistance to and tolerance of infection. In Trinidad, 'Ford Hook' ($v_1v_1V_2V_2$) plants are virtually always infected long before their period of useful production is over and there is every reason to replace them by the resistant 'Puerto Rico Wonder'. The vigor and long bearing life of 'Hot Yellow' is surely at least a partial consequence of its resistance to virus. (The situation is complicated by the fact that it is also resistant to attack by *Cercospora capsici* which is responsible for some defoliation of FH, LBH and PRW). It is interesting that V_1 survives in this strain even though $V_1v_2v_2$ plants,

if infected, are severely handicapped; the inference must be that v_2v_2, by reason of its control of infection, is of crucial importance in conferring field resistance, thus protecting the sublethal V_1 from elimination.

The symptoms of the disease described from Puerto Rico do not exactly agree with those known in Trinidad but the fact that 'Puerto Rico Wonder', bred in the island, is also resistant to the Trinidad virus is good evidence that the disease is indeed the same, minor differences in symptoms notwithstanding. This, the problem of identifying a virus disease, is a recurrent one (especially in a family such as the Solanaceae) and the genes described here offer a useful means of diagnosis (cf. HOLMES 1958).

Observations recorded above suggest that susceptible (i.e. V_2 and V_1V_2) genotypes occur and may be general in *Capsicum annuum;* but that other Capsicum species are predominantly resistant (v_1v_2 or V_1v_2). This is, at present, only a suggestion for clearly a much wider survey of botanically known materials is required.

Of the three virus diseases of Capsicum of which the genetics of host reaction has been investigated, one provides an instance of dominant resistance (tobacco mosaic—references above), one an instance of recessive resistance (tobacco etch virus—GREENLEAF 1956) and one an instance of complementary recessive resistance (pepper vein-banding virus—the present work). All three systems offer useful material alike to the plant breeder and to the pathologist in search of diagnostic test plants.

SUMMARY

Two loci control reaction to vein-banding virus in pepper (Capsicum) seedlings. There is no evidence of linkage between the two loci concerned. The reactions are: V_1V_2—easily infected, vein-necrosis, leaf abscission and death; $V_1v_2v_2$—resistant to infection, vein-necrosis, mosaic, partial abscission, stunted; $v_1v_1V_2$—easily infected, severe mosaic, poor growth; $v_1v_1v_2v_2$—resistant to infection, mild mosaic, good growth. Dominance of V_1 is incomplete and this, together with resistance to infection conferred by v_2v_2, causes some difficulties in classification.

ACKNOWLEDGMENTS

The writers are indebted to MR. W. T. DALE for supplying the original stocks and for help in the earlier phases of the experiments; to DR. F. J. SIMMONDS for the gift of seeds of 'Hot Yellow'; to DR. P. G. SMITH, of the University of California, for much useful advice by correspondence and for the gift of seeds of pepper species; and to PROFESSOR K. MATHER for valuable advice on the problem of estimation of misclassification parameters.

LITERATURE CITED

ADSUAR, J., 1940 Pepper. Ann. Rep. Agr. Exptl. Sta. Puerto Rico. 1939–40: 49–50.

DALE, W. T., 1954 Sap transmissible mosaic diseases of Solanaceous crops in Trinidad. Ann. Appl. Biol. **41**: 240–247.

GREENLEAF, W. H., 1956 Inheritance of resistance to tobacco etch virus in *Capsicum frutescens* and in *Capsicum annuum*. Phytopathol. **46**: 371–375.

HEISER, C. B., and P. G. SMITH, 1948 Observations on another species of cultivated pepper, *Capsicum pubescens* R. and P. Proc. Am. Soc. Hort. Sci. **52**: 331–335.

1953 The cultivated Capsicum peppers. Econ. Botany **7**: 214–227.

HOLMES, F. O., 1934 Inheritance of ability to localize tobacco mosaic virus. Phytopathol. **24**: 984–1002.

1937 Inheritance of resistance to tobacco mosaic disease in the pepper. Phytopathol. **27**: 637–642.

1958 A single gene resistance test for viral relationship as applied to strains of spotted wilt virus. Virology **5**: 382–390.

KIKUTA, K., 1947 Mosaic resistance in peppers. Bienn. Rep. Univ. Hawaii Agr. Exptl. Sta. 1944–46: 144–145.

LUTES, D. D., 1954 Inheritance of resistance to systemic tobacco mosaic infection in pepper. Dissertation Abstr. **14**: 9188. Pl. Br. Abstr. **25**: 2288.

MATHER, K., 1938 *The Measurement of Linkage in Heredity*. Methuen. London.

PEREZ, J. E., and J. ADSUAR, 1955 Antigenic relationship between Puerto Rican pepper mosaic virus and a strain of potato virus Y. J. Agr. Univ. Puerto Rico **39**: 165–167.

RIOLLANO, A., J. ADSUAR, and A. RODRIGUEZ, 1948 Breeding peppers resistant to a Puerto Rican type of mosaic. Proc. Am. Soc. Hort. Sci. **51**: 415–416.

ROQUE, A., and J. ADSUAR, 1941 Studies on the mosaic of peppers (*Capsicum frutescens*) in Puerto Rico. J. Agr. Univ. Puerto Rico **25**: 40–50.

SMITH, P. G., and C. B. HEISER, 1951 Taxonomic and genetic studies on the cultivated peppers, *Capsicum annuum* L. and *C. frutescens* L. Am. J. Botany **38**: 362–368.

SMITH, P. G., C. M. RICK, and C. B. HEISER, 1951 *Capsicum pendulum* Willd., another cultivated pepper from South America. Proc. Am. Soc. Hort. Sci. **57**: 339–342.

ASEXUAL SELECTION IN NEUROSPORA CRASSA[1]

ROWLAND DAVIS[2]

Biological Laboratories, Harvard University, Cambridge, Massachusetts

Received April 27, 1959

HETEROCARYOSIS, the association of genetically different nuclei in a single cytoplasmic unit, has been recognized in asexually reproducing fungi as a mechanism of natural variation since the work of HANSEN and SMITH (1932) on the "dual phenomenon." They discovered that wild isolates of *Botrytis cinerea* were unstable upon single conidial transfer, the wild isolate giving rise to two stable "conidial" and "mycelial" strains, as well as the unstable, intermediate wild type. Later investigations (HANSEN 1938) showed that this behavior was widespread among the Fungi Imperfecti. In 1942, HANSEN and SNYDER discovered the dual phenomenon in the sexually reproducing Ascomycete, *Hypomyces solani*. By standard genetic tests, it was shown that the "conidial" and "mycelial" characters were controlled by a single pair of alleles, and that the dual phenomenon was doubtless the result of the assortment of two different nuclear types of a heterocaryon during the formation of multinucleate conidia.

The investigations of DODGE (1942), and of BEADLE and COONRADT (1944) with various mutant strains of Neurospora species have revealed a great deal concerning the physiological consequences of heterocaryosis. In most cases, it was found that heterocaryotic mycelia, constituted of two nuclear types carrying non-allelic mutations would display a normal phenotype. They felt that the mutant gene carried by one nuclear type was compensated for by the action of its wild type allele in the other nuclear type. Though not strictly comparable with dominance in diploid systems, the complementary action of wild type genes over their mutant alleles in heterocaryons indicated a dominant-recessive relationship.

The occurrence of the dual phenomenon in nature and the experimental investigations of heterocaryosis indicate that filamentous fungi possess a more adaptable genetic system than do unicellular haploid organisms. Because many species of fungi reproduce by multinucleate conidia, a heterocaryotic condition may be maintained not only during the growth of mycelia, but also in their asexual reproduction. The plasticity of such a system would be somewhat akin to that of diploids, because a reserve of variability could accumulate in a nuclear population by mutation, shielded from adverse selection by more advantageous genomes.

[1] Work performed while the author was a U.S. Public Health Service Predoctoral Research Fellow of the National Cancer Institute. Presented to the Department of Biology, Harvard University as a portion of a dissertation in partial fulfillment of the requirements for the degree of Ph.D.

[2] Present address: Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California.

The changes in the nuclear proportions of heterocaryons during conidial transfers, therefore, may be investigated, and such a study may be used to construct a model of natural selection in fungi which reproduce asexually by means of multinucleate conidia. Asexual reproduction in *N. crassa* involves uni- and multinucleate conidia, among which nuclei of different types may be distributed almost randomly (PROUT, HUEBSCHMAN, LEVENE and RYAN 1953; ATWOOD and MUKAI 1955; and KLEIN 1958). A heterocaryon may therefore produce conidia of three classes; two of which contain only one type of nucleus (homocaryotic) and one of which contains both (heterocaryotic). With a heterocaryotic system constituted of two nutritionally deficient nuclear types, it is possible to select against a homocaryotic conidial type by omitting its nutritional requirement. By studying the changes of nuclear proportions over many serial conidial transfers, the factors governing these changes may be determined. The experiments reported here were designed to elucidate these factors and to indicate the extent to which nuclear selection in asexually reproducing heterocaryons of *N. crassa* is comparable with the pattern of genic selection in populations of sexually reproducing diploid organisms.

MATERIALS AND METHODS

The homocaryotic strains of *N. crassa* from which the heterocaryons were synthesized were derived from single ascospore isolates of the genotypes *pan, al-1 A* (5531, 4637; pantothenic acid, albino) and *nic-2, al-2 A* (4540, 15300; nicotinic acid, albino). The mutations are described in the index of BARRATT, NEWMEYER, PERKINS and GARNJOBST (1954).

The medium used for the experimental transfers of the heterocaryons was the minimal medium of RYAN (1950). This will be referred to as "standard medium." The medium used in plating conidia for estimations of nuclear ratios was a modified form of that of WESTERGAARD and MITCHELL (1947), and contained one percent sorbose and 0.1 percent sucrose. Either medium, when unsupplemented, will be described as "minimal." Where nutritional supplements were appropriate, they were added in the following concentrations: calcium pantothenate, 2 μg per ml medium; nicotinic acid, 2 μg per ml; nicotinamide, 2 μg per ml; and l-lysine, 50 μg per ml.

The heterocaryons of *pan, al-1* and *nic-2, al-2* used in the experiments were formed by the method of PITTENGER, KIMBALL, and ATWOOD (1955). Heterocaryons constituted of these nuclear types grow without either growth supplement at a rate indistinguishable from that of wild type. Mycelia of this type form three types of conidia: *pan, al-1* homocaryons, *nic-2, al-2* homocaryons, and heterocaryons, containing both nuclear types. The present experiments were designed to select against one or the other of the homocaryotic conidial types during conidial transfers. This was accomplished by establishing eight heterocaryons and subsequently transferring four of them serially on pantothenate supplemented standard medium (PAN lines), and four of them on nicotinic acid supplemented medium (NIC lines). Selection was maintained over many serial transfers on

agar slants in 18 × 150 mm culture tubes, and the changes in nuclear ratio were recorded. The term *transfer* will be used for the most part; for clarity in some cases, the term *generation* will refer to the heterocaryon arising from a given transfer. The regular transfers of the lines were performed by taking a suspension of conidia from a heterocaryon, filtering it through glass wool, and diluting it to a concentration of 1000 to 6000 conidia per ml. 0.1 ml of the dilution was pipetted evenly over the slants. After four days, the resulting mycelium had developed a rich conidial mass, and the procedure was repeated.

At each transfer, determinations of the frequencies of the three conidial types from each heterocaryon were made in order to estimate the nuclear proportions. This was done by plating aliquots of the conidial suspension used for transfers in triplicate on three types of media: minimal plating medium + lysine, minimal + pantothenate + lysine, and minimal + nicotinamide + lysine. (Lysine was previously found to improve the germination of conidia in this medium.) By appropriately subtracting colony counts on minimal medium from those on supplemented media, the frequencies of the three conidial types were obtained. At disparate nuclear ratios, where it was expected that heterocaryotic conidia and homocaryotic conidia of one type would be rare, the number of conidia plated in minimal and in medium supplemented to select the rare homocaryon was increased tenfold. This allowed a more accurate determination of the proportions of minority conidial types.

The average number of nuclei per conidium, which in most cases lies between two and three, was determined at each transfer by counting 500 stained conidia (HUEBSCHMAN 1952). The conidia of one of the PAN lines and of one of the NIC lines were sampled at each transfer, and the average nuclear number was used in calculating the nuclear ratios of the other PAN and NIC lines. In later experiments, the average nuclear number used was 2.60, an average value found in the first experiments.

Estimations of the nuclear proportions, using the conidial frequencies and average nuclear number per conidium were made according to the formula of ATWOOD and MUKAI (1955; see below).

THEORY AND NOTATION

Although a refined method for the estimation of nuclear proportions has been described (PROUT *et al.* 1953), it assumes a random distribution of nuclear types in the conidia of heterocaryons and its usefulness is limited by the observed departure from randomness in some cases (PROUT *et al.* 1953; ATWOOD and MUKAI 1955; KLEIN 1958), and by the complexity of applying it. More recently, ATWOOD and MUKAI (1955) have given a simpler formula which takes into account departures from randomness, and which gives approximately the same values as that of PROUT *et al.* Because random distribution of nuclear types in conidia is not an important consideration here, and since the application of ATWOOD and MUKAI's formula is justifiable in more cases, the latter is used as the basis of the theory stated below.

ATWOOD and MUKAI derive their formula for the estimation of nuclear proportions in conidia as follows (paraphrased): Let the frequency of the *pan, al-1* homocaryons be represented by a, that of the *nic-2, al-2* homocaryons be represented by b, and that of the heterocaryons be represented by r. Thus, $a + b + r = 1$. If N is the total number of conidia in the samples added to each plate, and if $\overline{n}$ is the average nuclear number, $N\overline{n}$ is the total nuclear population to be considered. In heterocaryotic conidia, at least one nucleus of each type is present. Thus the known number of *pan, al-1* nuclei in the population is Nr. The nuclear population in homocaryotic conidia plus those in excess of the two known in each heterocaryotic conidium is $N\overline{n} - 2Nr$. It is then assumed that in this remaining population, the ratio of the two types of nuclei is the same as that of the corresponding homocaryotic conidial types. The proportion of *pan, al-1* homocaryons among homocaryotic conidia is $a/(1-r)$. To find the number of *pan, al-1* nuclei in the total nuclear population, $N\overline{n} - 2Nr$ is multiplied by $a/1-r$ and added to Nr. This number is then divided by the total nuclear population, $N\overline{n}$, to give the approximate proportion p, of *pan, al-1* nuclei:

$$p = \frac{r(1-r) + a(\overline{n}-2r)}{\overline{n}(1-r)} \qquad \text{(I)}$$

An essential feature of this formula is the simplifying assumption that all three conidial types have equal average nuclear numbers. This is justified to some extent by the observed tendency for more homocaryotic conidia to be formed, at the expense of heterocaryotic conidia, than would be expected on the basis of random distribution of nuclei in conidia.

ATWOOD and MUKAI have not gone beyond the use of this formula in the estimation of nuclear proportions. If this formula is valid, and if the conidial frequencies found by plating represent the frequencies of conidia transferred during the selection procedure outlined above, it should be possible to predict the value of p for the next generation. Assuming *nic-2 al-2* cells to be inviable on medium containing only calcium pantothenate, the proportion of the conidial population which is able to grow is $a + r$. Because the average nuclear numbers of all types of conidia are assumed to be equal, $a + r$ also represents the proportion of the nuclear population which resides in conidia capable of growth. If the proportion of *pan, al-1* nuclei of a given heterocaryon be divided by $a + r$, one obtains the proportion of *pan, al-1* nuclei in conidia able to grow on pantothenate. If it is further assumed that equal division rates of the two nuclear types of these conidia takes place (PITTENGER and ATWOOD 1956), then this fraction will equal the frequency of *pan, al-1* nuclei in the next generation. This value, predicted from the previous generation by assuming that *nic-2, al-2* conidia cannot grow, will be denoted

$$p' = \frac{p}{a+r} \quad . \qquad \text{(II)}$$

The equivalent expression for selection in favor of the *nic-2*, *al-2* nuclear type is:

$$(1-p)' = \frac{(1-p)}{b+r} \quad . \tag{III}$$

If one assumes that both homocaryotic conidial types are selected against in a given transfer, and that only heterocaryotic conidia contribute to the resulting heterocaryon, one may predict the proportion of *pan*, *al-1* nuclei in a heterocaryon grown under these conditions. Nr represents the number of *pan*, *al-1* nuclei known to be present in heterocaryotic conidia; to this must be added the number of nuclei in excess of the two known (i.e., $N\bar{n}r - 2Nr$), multiplied by the proportion, $a/(1-r)$, of *pan*, *al-1* nuclei among them. This sum is then divided by the total number of nuclei in heterocaryotic conidia, $N\bar{n}r$, to obtain the proportion of *pan*, *al-1* nuclei. Thus the proportion of *pan*, *al-1* nuclei in heterocaryotic conidia will be:

$$p'' = \frac{Nr + \dfrac{a(N\bar{n}r - 2Nr)}{1-r}}{N\bar{n}r} \tag{IV}$$

$$= \frac{(1-r) + a(\bar{n}-2)}{\bar{n}(1-r)} \quad , \tag{V}$$

and p'' will represent the predicted frequency of *pan*, *al-1* nuclei of a heterocaryon derived from heterocaryotic conidia only. The predicted frequency, $(1-p)''$, of *nic-2*, *al-2* nuclei under the same conditions will be:

$$(1-p)'' = \frac{(1-r) + b(\bar{n}-2)}{n(1-r)} \quad . \tag{VI}$$

It may be seen that as long as the average number of nuclei per conidium is nearly two, the last two formulae will approximate 0.50. This, however, is somewhat unrealistic when it is realized that the value of $\bar{n}$ for heterocaryotic conidia will in all probability be greater than 2, this being the lower limit of the nuclear frequency distribution for this conidial type. A correction of this feature, made by disregarding uninucleate conidia in computing $\bar{n}$ would modify it by only ten percent in most cases. The effect on the values of p and $1-p$ is almost negligible for the present analysis. Now, as $\bar{n}$ becomes larger, the values p'' and $(1-p)''$ of selected lines in which only heterocaryotic conidia are able to grow will tend toward 0.50. The only stable value is 0.50, theoretically, since the two formulae (V and VI) are dominated by the 1:1 ratio of the two nuclear types essential to every heterocaryotic conidium.

A general formula may now be derived, from which variable contributions of one homocaryotic conidial class selected for may be found, assuming the other homocaryotic class to be completely selected against.

If $N\bar{n}a$ is the total number of nuclei in conidia homocaryotic for *pan*, *al-1*, and if x is taken to be the proportion of these nuclei which contribute to the next generation, then $N\bar{n}ax$ is the number of these nuclei which contribute to the next

generation. By adding the quantity $N\bar{n}ax$ to both numerator and denominator of the right hand side of Formula IV, we obtain, after simplifying:

$$p_{t+1} = \frac{r(1-r) + a(\bar{n}r - 2r) + \bar{n}a(1-r)x}{\bar{n}(1-r)\ (r + ax)} \quad , \qquad \text{(VII)}$$

where t is the generation from which the values of a, r, and $\bar{n}$ are taken, and where p_{t+1} is the value of p in the next. This expression may be used to find the proportion of *pan, al-1* homocaryons which contribute to the latter generation, relative to the heterocaryotic type, by solving for x. The proportion of the heterocaryotic type which contributes is assumed to be 1.00. The formula may be modified to describe the relative contribution of *nic-2, al-2* homocaryons if *pan, al-1* homocaryons are absolutely selected against, as follows:

$$(1-p)_{t+1} = \frac{r(1-r) + b(\bar{n}r - 2r) + \bar{n}b(1-r)y}{\bar{n}(1-r)\ (r + by)} \qquad \text{(VIII)}$$

where y is the proportion of *nic-2, al-2* conidia that grow, or where y is the extent to which all *nic-2, al-2* conidia contribute to the final nuclear population.

Now, if $x = 0$, formula VII reduces to the value of p'', and if $x = 1$, formula VII reduces to that of p'. If the actual value of p found in a given generation is used as p_{t+1}, the magnitude of x, the selective value of the homocaryotic conidial class, may be obtained.

To summarize: Given certain conditions, it is possible to predict the frequencies of nuclei in a heterocaryon by analyzing the conidial population from which the heterocaryon is derived. The predicted frequencies may be obtained by assuming that *pan, al-1* homocaryons and heterocaryons grow and contribute equally to the resulting heterocaryon (prediction p'), that *nic-2, al-2* homocaryons and heterocaryons grow equally well (prediction $(1-p)'$), or that only heterocaryons grow (predictions p'' and $(1-p)''$). These predictions may be compared graphically to the observed value of p or $(1-p)$ of the heterocaryons at each generation during selection. Furthermore, inequalities in the relative contribution, or selective value, of the heterocaryotic and one homocaryotic conidial class may be found by analyzing the data with Formulae VII and VIII.

RESULTS

Reliability of methods

Two *pan, al-1* + *nic-2, al-2* heterocaryons were analyzed in quadruplicate in order to estimate the range of variation in the nuclear ratio determinations. From each heterocaryon, four platings were made to determine the frequencies of the three conidial types, and four samples of conidia were stained for the determination of average nuclear numbers. The value of p for the first heterocaryon ranged from 0.92–0.95 (std. dev. 0.013), and for the second, from 0.70–0.74 (std. dev. 0.016). The data indicate that values of p are consistent within about 0.03 (two std. dev's.).

Selection on mixtures of homocaryotic conidia

Since homocaryotic conidia on unsupplemented medium can develop short germ tubes, heterocaryosis between conidia selected for and those selected against might be expected to occur by the anastomosis of these germ tubes. To test the extent of this possible heterocaryosis, 0.1 ml aliquots of a 1:1 mixture of *pan, al-1* and *nic-2, al-2* conidia containing 10^5 conidia per ml were placed on slants of standard minimal medium, minimal + pantothenate, and minimal + nicotinic acid. This duplicated as nearly as possible the conditions prevailing during the selection experiments with regard to media and the distribution of conidia on the slants.

In the tubes of minimal medium, no growth took place. Conidia from the mycelia developing in the supplemented tubes were plated on minimal and both singly supplemented plating media to test for the presence of the nuclear type selected against. No nuclei of these types expressed themselves in platings of 5×10^4 conidia. It may be concluded that in these conditions, heterocaryotic associations involving conidia selected against do not occur to any significant extent.

Changes of nuclear proportions during selection

The changes in nuclear proportions during selection were gradual. In the PAN lines, where *nic-2, al-2* homocaryons were selected against, the frequency, p, of the *pan, al-1* nuclear type increased from about 0.70 to about 0.95 or more in eight transfers. Selection was maintained further in two of these lines, PAN-1 and PAN-4. *Nic-2, al-2* nuclei disappeared from PAN-1 five transfers later, and still remained in PAN-4 ten transfers later, at which point the experiment was terminated. The data are shown in Table 1 and in Figure 1.

The frequencies expected on the basis of p' and p'' are also shown in Table 1 and in Figure 1. In the figure, the points on the broken or dotted lines are the predicted values for points at the same transfer on the "observed" (solid) curve. The predicted values have been connected to reveal more clearly any consistent deviations from the observed values. Statistical comparison of single observed and expected values was deemed unsuitable because of the unknown extent of the variation of individual estimates of p.

The points on the p'' curve show a large and consistent deviation from those observed. The assumption basic to this prediction, namely that only heterocaryotic conidia grow, clearly does not describe the course of selection. In the case of the p' prediction, the points agree very well with those observed. However, the agreement is meaningful only before the frequency of *pan, al-1* nuclei reaches 0.95. Above this range, the quantification of the minority homocaryotic conidial type becomes increasingly difficult, and the variation in nuclear proportions from generation to generation becomes very slight. Thus the frequencies of *pan, al-1* nuclei in the PAN lines, before they reach the value of 0.95, may be compared to the prediction p' by pooling the data for all four lines, and estimating (1) the average value of the differences of p and p', and (2) the standard deviation of this difference. The average difference, that is, the sum of the differences $(p-p')$

TABLE 1

PAN lines: observed nuclear frequencies (p) *and those predicted* (p′ *and* p″) *during the course of selection*

Transfer	PAN–1 p	p'	p''
0	.66	..	..
1	.80	.81	.56
2	.86	.86	.59
3	.88	.89	.51
4	.91	.91	.54
5	.95	.94	.55
6	.96	.95	.64
7	.96	.96	.68
8	.99	.97	.58
9	.99	.99	.52
10	.98	.99	.59
14	.996	..	..
15	1.00	..	..

Transfer	PAN–2 p	p'	p''
0	.75	..	..
1	.87	.81	.56
2	.84	.87	.68
3	.90	.89	.51
4	.92	.93	.54
5	.94	.93	.56
6	.90	.96	.62
7	.96	.95	.66
8	.95	.97	.58

Transfer	PAN–3 p	p'	p''
0	.72	..	..
1	.86	.78	.62
2	.86	.87	.59
3	.89	.90	.51
4	.90	.92	.54
5	.87	.92	.56
6	.92	.90	.61
7	.94	.93	.67
8	.98	.95	.58

Transfer	PAN–4 p	p'	p''
0	.76	..	..
1	.83	.82	.58
2	.88	.85	.64
3	.91	.89	.51
4	.96	.91	.54
5	.95	.96	.56
6	.97	.98	.61
7	.98	.98	.68
8	.98	.98	.58
9	.98	.99	.52
10	.98	.98	.59
14	.994	..	..
16	.9994	..	..
17	.9994	..	..
18	.9999	..	..
19	.9994	..	..

divided by the 21 comparisons made, is + 0.0024. This indicates that the values of p conform very well to the prediction p', and that the deviations are not consistent in their direction. The standard deviation of the difference, using 0.00 as the average, is 0.032; this value denotes the general magnitude of the differences of p and p' and is not significantly different from the range of variations found in the replicate platings of individual heterocaryons. The simple assumptions involved in the p' prediction, therefore, may be used to describe the course of selection in the PAN lines.

In all four of the NIC lines, in which *pan, al-1* homocaryons were absolutely selected against, the frequency of the *nic-2, al-2* nuclei, $1-p$, increased from as low as 0.42 to 0.50–0.58, in which range it remained in subsequent transfers with

no consistent departures. The data are given in Table 2 and in Figure 2, together with the $(1-p)'$ and $(1-p)''$ predictions.

The relationship of the observed values of $1-p$ to the two predictions in the

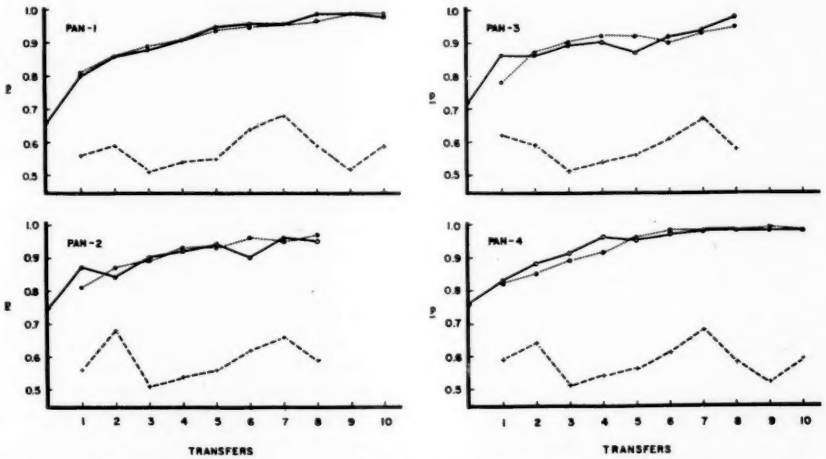


FIGURE 1.—Changes in the observed (p) and predicted (p' and p'') frequencies of *pan, al-1* nuclei taking place in the PAN lines during the course of selection. Key: p, solid line; p', dotted line; p'', dashed line.

TABLE 2

NIC lines: observed nuclear frequencies (1–p), and those predicted ((1–p)' and (1–p)'') during the course of selection

Transfer	NIC–1 1–p	$(1-p)'$	$(1-p)''$	Transfer	NIC–2 1–p	$(1-p)'$	$(1-p)''$
0	.47	..	..	0	.42	..	..
1	.50	.71	.49	1	.49	.75	.49
2	.54	.72	.50	2	.57	.71	.50
3	.58	.70	.51	3	.58	.71	.53
4	.58	.75	.52	4	.53	.70	.52
5	.58	.77	.53	5	.52	.72	.51

Transfer	NIC–3 1–p	$(1-p)'$	$(1-p)''$	Transfer	NIC–4 1–p	$(1-p)'$	$(1-p)''$
0	.46	..	..	0	.53	..	..
1	.52	.72	.49	1	.48	.77	.49
2	.54	.72	.50	2	.51	.74	.54
3	.53	.73	.52	3	.54	.72	.50
4	.55	.72	.51	4	.57	.76	.51
5	.50	.70	.52	5	.57	.74	.53

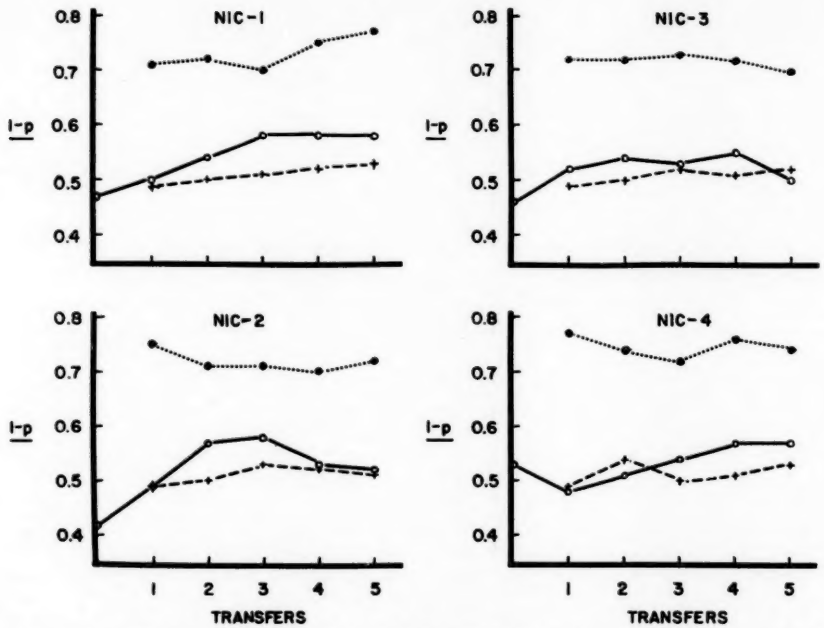


FIGURE 2.—Changes in the observed $(1-p)$ and predicted ($(1-p)'$ and $(1-p)''$) frequencies of *nic-2*, *al-2* nuclei taking place in the NIC lines during the course of selection. Key $1-p$, solid line, $(1-p)'$, dotted line; $(1-p)''$, dashed line.

NIC lines is quite different from that in the PAN lines. In the NIC lines, $1-p$ deviates widely from the prediction $(1-p)'$, and tends to follow the prediction $(1-p)''$ instead. By comparing $1-p$ to $(1-p)''$ in a manner similar to that used in the case of the PAN lines, the average difference between $1-p$ and $(1-p)''$ is $+0.029$. This indicates that the observed values lay, on the average, consistently 0.03 above those predicted according to the assumption that only heterocaryotic conidia grow. The standard deviation of the difference, using 0.03 as the average, is 0.03, a figure compatible with the range of variation found in platings of individual heterocaryons.

Because the only stable value of $(1-p)''$, theoretically, is 0.50, and because the value of $1-p$ appears to be relatively stable at approximately 0.54, it is tentatively concluded that in the NIC lines, conidia homocaryotic for *nic-2*, *al-2* nuclei are contributing slightly to the final nuclear population at each transfer. In light of the fact that 0.54 is the average value of $1-p$ for the NIC lines throughout the experiments, it is possible to estimate the proportion of *nic-2*, *al-2* homocaryons, γ, contributing to each generation. This is done by choosing the values of a, b, r, $\overline{n}$, and $(1+p)_{t+1}$ for specific transfers, substituting these in Formula VIII, and solving for γ. In Table 3 are presented the results of such an analysis for three

TABLE 3

Representative values of y: *Data for generation 2 of the NIC lines 1, 3, and 4, with observed and expected values of 1–*p *for generation 3, and calculated values of* y*

Line	Transfer	r	a	b	$\bar{n}$	$(1-p)_2$	$(1-p)''_3$ Expec.	$(1-p)_3$ Observ.	γ
NIC–3	2 to 3	.414	.259	.327	2.64	.54	.52	.53	0.04
NIC–4	2 to 3	.414	.292	.294	2.64	.51	.50	.54	0.14
NIC–1	2 to 3	.482	.228	.290	2.64	.54	.51	.58	0.24

* r, a, and b are the frequencies of heterocaryons, *pan, al–1* homocaryons and *nic–2, al–2* homocaryons, respectively. $\bar{n}$ is the average number of nuclei per conidium. See text for further explanation.

lines in the transfer from the second to the third generation. The transfers are chosen according to the differences between the observed and the expected values at the third generation. The first pair of values, differing by 0.01, gives $\gamma = 0.04$, the second, differing by 0.04, gives $\gamma = 0.14$, and the third, differing by 0.07, gives $\gamma = 0.24$. To represent the general behavior exhibited by the NIC lines, the data of the third case listed may be taken, with the provision that $(1-p)_3$ equal 0.54. The latter value, the average of $1-p$ throughout, is 0.03 above the $(1-p)''$ prediction. The value of γ using this value of $1-p$ is 0.09, which is a rough approximation of the extent to which *nic-2, al-2* conidia contribute to each generation if $1-p$ is maintained at an equilibrium value of 0.54.

To summarize: (1) In the PAN lines, the selective value of *pan, al-1* conidia, x, is 1.00, relative to the heterocaryotic conidial type. This characteristic leads to the reduction in frequency and the virtual disappearance of the *nic-2, al-2* nuclear type after 15 to 20 transfers. (2) In the NIC lines, the heterocaryotic conidia appear to be the main contributors to each generation, and the selective advantage of the *nic-2, al-2* conidia is approximately 0.09, relative to that of the heterocaryotic conidia. This leads to the establishment of an equilibrium of the nuclear proportions which favors the *nic-2, al-2* nuclear type slightly.

The selective disadvantage of nic-2, al-2

To disclose more clearly the apparent selective disadvantage of *nic-2, al-2* conidia as compared to the heterocaryotic conidia in the NIC lines, homocaryotic cultures of *nic-2, al-2* and *pan, al-1* were isolated during the selection experiments. These were compared to the heterocaryon and to each other in various ways.

1. The growth rates of *pan, al-1* on pantothenate supplemented medium, of *nic-2, al-2* on nicotinic supplemented medium, and of the heterocaryon on both types of media were found to be indistinguishable, by the tube method of determining mycelial growth rates (RYAN, BEADLE and TATUM 1943). In a second experiment, the nuclear ratios of three heterocaryons at the beginning and end of 450 mm growth tubes were determined. In no case was the difference in the value of p more than 0.03 at the beginning than at the end. This result indicated that the rates of increase of the nuclear types were essentially equal during the growth of these heterocaryons (PITTENGER and ATWOOD 1956), and that the

disadvantage of *nic-2, al-2* in the NIC lines could not be explained in terms of nuclear competition in growing heterocaryotic mycelia.

2. The early development of mycelia from conidial inocula was then studied. Slants of standard minimal and supplemented media, comparable to those used in the selection experiments, were inoculated with 0.1 ml of conidial suspension of *pan, al-1, nic-2, al-2*, or a heterocaryon. After 40 hours, the growth in tubes inoculated with *nic-2, al-2* conidia in numbers similar to those used in transferring the NIC and PAN lines was just visible as a thin network of hypae covering the slant. After the same interval, inocula of the same numbers of conidia of both *pan, al-1* and the heterocaryon had given rise to rich mycelia with aerial mycelium. This result is consistent with the indication from experiments with the NIC lines that the growth of heterocaryotic conidia swamps that of *nic-2, al-2* homocaryons, whereas in the PAN lines, *pan, al-1* and heterocaryotic conidia contribute equally to the next generation.

This same method was used to test the effects of changes in the medium upon the establishment of mycelia. In a second series of experiments, the early growth of the heterocaryon and of *nic-2, al-2* from conidial inocula was compared on combinations of one or two percent sucrose and nicotinic acid or nicotinamide. The medium used in the selection experiments, containing one percent sucrose and nicotinic acid, was found to be least favorable for the growth of *nic-2, al-2*. Growth proceeded somewhat faster on two percent sucrose and nicotinic acid, and the substitution of nicotinamide for nicotinic acid resulted in a developmental rate comparable to that of the heterocaryon. The growth of the heterocaryon did not differ significantly with these substitutions in the medium. The differential effect of nicotinic acid and nicotinamide has been mentioned by BARRATT *et al.* (1954). This information will be utilized in the further selection experiments reported below.

3. From the preceding experiments, there appears to be no competition between *pan, al-1* and *nic-2, al-2* nuclei when they are associated in heterocaryons, but the early growth of *nic-2, al-2* conidial inocula is retarded, compared to that of other inocula, on the medium used in the selection experiments. It is postulated then, that selection against *nic-2, al-2* nuclei in the NIC lines operates before the fusion of germ tubes and mycelium developing from *nic-2, al-2* and heterocaryotic conidia. A more direct test of this assumption was made in the following experiment.

It has been shown that the early development of mycelia derived from *pan, al-1* and heterocaryotic conidia takes place at approximately the same rate. By inoculating slants containing both pantothenate and nicotinic acid with a 1:1 mixture of *pan, al-1* and *nic-2, al-2* conidia, and determining the nuclear ratio of the resulting heterocaryon, the extent to which *nic-2, al-2* nuclei are selected against may be found. Because the conidia of *pan, al-1* and *nic-2, al-2* are in a 1:1 ratio in the present experiment, immediate fusion of their germ tubes would be expected to result in equal proportions of the two nuclear types in the heterocaryon. As the concentration of the 1:1 mixture is increased, it is expected that a

1:1 nuclear ratio in the resulting heterocaryon will be approached. As fewer conidia are used in the inoculum, more growth may occur before fusion, and selection would be expected to operate to a greater extent.

The result of this experiment was that when 10^6 conidia were used, $1-p = 0.47$; 10^5 conidia: $1-p = 0.54$; 10^3 conidia: $1-p = 0.29$; 10^2 conidia: $1-p = 0.15$ (the 10^4 tube was lost). These nuclear ratios indicate that selection against *nic-2, al-2* becomes more pronounced as the concentration of conidia decreases. It is concluded that selection against *nic-2, al-2* in the NIC lines operates before the fusion of growth from conidia used in the transfers.

Further selection experiments

The following brief selection experiments were made to check the previous findings, to test the effect of different initial proportions, and to test the effect of different media, all with a view to testing further the hypotheses presented above.

1) A heterocaryon constituted of 0.21 *pan, al-1* and 0.79 *nic-2, al-2* nuclei was serially transferred on pantothenate supplemented medium for six generations. The value of p rose to 0.97 in six transfers, and was reasonably well in accord with the p' prediction (Figure 3a).

2) A heterocaryon containing 0.80 *nic-2, al-2* nuclei was transferred on nicotinic acid supplemented medium for seven generations. The proportion of *nic-2, al-2* nuclei declined within three generations to 0.51, and followed the $(1-p)''$ prediction rather closely. After these three transfers, the medium was changed to contain two, instead of one percent sucrose. From this time the observed curve of $1-p$ consistently rose, departing from the $(1-p)''$ prediction, and followed a middle course between $(1-p)'$ and $(1-p)''$. The data are shown graphically in Figure 3b. The difference in the behavior of the heterocaryon on the two levels of sucrose may be understood in terms of the beneficial effect of added sucrose on the rate of mycelial development of *nic-2, al-2*.

3) A heterocaryon containing 0.55 *nic-2, al-2* nuclei was transferred for four transfers on nicotinamide supplemented medium. Here, the $1-p$ curve follows the $(1-p)'$ prediction (Figure 3c), and the final proportion of *nic-2, al-2* nuclei was 0.92. Nicotinamide appears to equalize the selective advantage of *nic-2, al-2* and heterocaryotic conidia, a finding also indicated by the effect of nicotinamide on the rate of mycelial establishment of *nic-2, al-2*.

4) A heterocaryon containing 0.17 *pan, al-1* nuclei was transferred for seven generations on minimal medium. In this case, the p curve follows p'' quite closely at first, but then tends to fluctuate slightly above 0.50 (Figure 3d). The p values average 0.023 above the p'' prediction. The discrepancy may represent a slight differential mortality of *nic-2, al-2* conidia over *pan, al-1* in plating for nuclear ratio, but the experiment indicates that p will stabilize around 0.50 if heterocaryotic conidia are the sole contributors to each generation (See Theory and Notation).

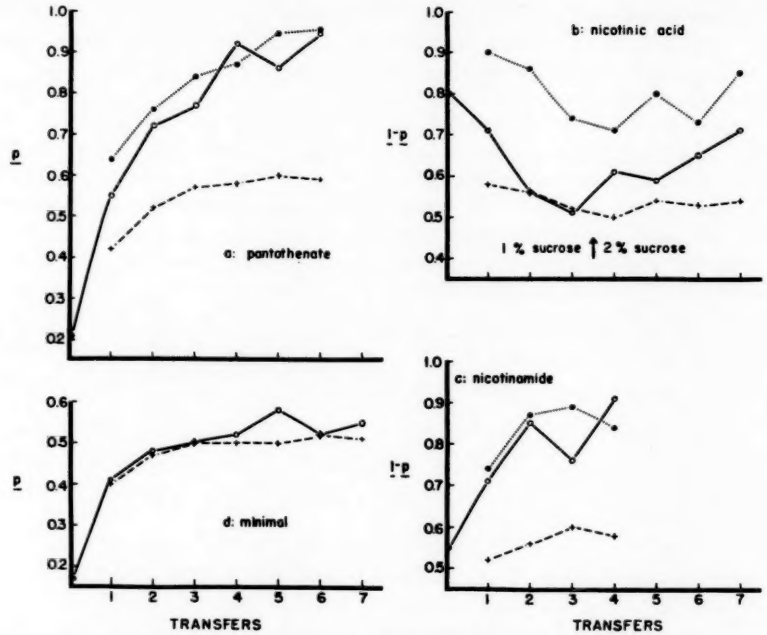


FIGURE 3.—Changes in nuclear frequencies taking place during selection of heterocaryons on different media. Key: p or $1—p$, solid lines; p' or $(1—p)'$, dotted lines; p'' or $(1—p)''$, dashed line.

DISCUSSION AND CONCLUSIONS

The foregoing experiments illustrate quantitatively the dampening effect of heterocaryosis on selection against "disadvantageous" nuclear types. In the PAN lines, where the *nic-2*, *al-2* nuclear type was selected against, the loss of *nic-2*, *al-2* nuclei was rapid when it was present in high frequency, but may be maintained at low frequencies for considerable periods of time. The latter feature permits the heterocaryon to adapt readily to a reversal of nutritional conditions. In the NIC lines, where nicotinic acid is the supplement in the medium, both *pan*, *al-1* and *nic-2*, *al-2* nuclei are maintained in almost equal frequency. Here, under conditions where *nic-2*, *al-2* and heterocaryotic mycelia may grow at the same rate, *nic-2*, *al-2* homocaryons appear to be selected against almost as strongly as *pan*, *al-1* homocaryons, and heterocaryotic conidia are the main contributors to each generation. The slow rate of germination of *nic-2*, *al-2* homocaryons, as compared to the heterocaryons, appears to be a sufficient explanation of this finding.

The model which has been proposed has been tested by comparing predictions, based on simple assumptions, with the observed changes in nuclear proportions.

The predictions p' and $(1-p)'$ are based on the assumptions a) that conidia of the homocaryotic class selected for and of the heterocaryotic class develop at the same rate, b) that the two nuclear components of heterocaryotic mycelia divide at the same rate, c) that each conidial type have the same average nuclear number, and d) that there be no differential mortality of conidial types in testing for nuclear ratio that does not also prevail in the transfer procedure. The basis of the predictions p'' and $(1-p)''$ differ only in the first assumption, which is in this case modified to state that only heterocaryotic conidia develop.

The first assumption and its alternate may be modified quantitatively in the light of experimental data, as seen in the case of the NIC lines. The second assumption finds support in the line transferred on minimal medium (Figure 3d) and in the observed stability of nuclear proportions during mycelial growth. The third point, (c), is open to some question, being only partially justified (ATWOOD and MUKAI 1955). It is, however, a simplifying assumption which does not do much violence to the accuracy of the estimations and predictions. A modification of the model based upon the assumption of random association of nuclei in conidia, and the concomitant inequalities in nuclear number of the three conidial types would not affect the interpretations appreciably, except in the case of highly disparate ratios. All data are reasonably consistent with the last assumption, although no direct proof of it has been obtained.

The data, therefore, may be analyzed in terms of the model quite well. There is room for minor changes in the model, based upon more refined data, but its present form is the simplest that may be derived from the present knowledge of the system.

In the context of the experiments, in which one homocaryotic conidial type is absolutely selected against, the model allows one to determine the selective value (x or y) of the other homocaryotic conidial type in relation to the heterocaryotic type. This is a valid calculation only if it is known that the nuclei involved in heterocaryotic conidia and mycelia have in general the same division rates. Thus, when the homocaryotic conidial class selected for has a selective value equal to or greater than 1.00 (relative to the heterocaryotic type), the frequency of the corresponding nuclear type may reach the value of 1.00. However, if its selective value is anything less than 1.00, both types of nuclei will be maintained in a large population permanently under constant nutritional conditions. The equilibrium nuclear proportions are dictated by the relative selective value of the two viable conidial types. This is an inescapable feature of Formulae VII and VIII.

The formulae presented may be modified to conform to other selective conditions. For instance, if the heterocaryotic and one homocaryotic conidial types have equal selective values, and the third conidial type has a selective value between 0 and 1.00, the right hand sides of Formulae II and III may be changed to $\frac{p}{a+r+yb}$ and $\frac{1-p}{b+r+xa}$, respectively. Also, if both homocaryotic conidial types

have selective values less than that of the heterocaryotic type, Formulae V and VI may be expanded to take this into account.

Whether the model proposed here may be applied to similar patterns of selection in nature remains to be determined. The strains of *N. crassa* used here, although not representative of naturally occurring ones in their genetic heterogeneity, do illustrate the fate of nuclei bearing certain genes which are "recessive" in the sense of BEADLE and COONRADT (1944). The model would have to be elaborated further to take into account such factors as incomplete recessiveness and differences in the division rates of different nuclear types in heterocaryons.

A last point is the comparison of nuclear selection in *N. crassa* and the theoretical pattern of selection against a lethal gene in diploid populations. The two types of organisms are exactly comparable in the following particulars:

1) A "deleterious" element (gene or nucleus) of the population may have little effect when associated with an advantageous element, but may be lethal when not so associated.

2) The reduction in frequency of a deleterious element may be referred to the lack of fitness of the recessive homozygotes in one case, and of disadvantageous homocaryons in the other.

3) As the frequency of the deleterious element decreases, a greater proportion of them are associated with the advantageous element. The rate of disappearance of the deleterious element therefore becomes slower and slower with time.

4) If the "deleterious" element should, through altered environmental circumstances, come to have a greater selective value than its alternate, selection may restore the former to prominence rather quickly. The maintenance of variability is therefore of considerable significance in unstable environments.

The systems are different in these respects: In diploids, sexual reproduction is a process by which combinations of genes are made; these combinations are made in pairs. In *N. crassa*, asexual reproduction of a heterocaryon involves the association of nuclei. The number of nuclei in each conidium may vary from one to ten or more.

With these points in mind, one may graphically compare the process of selection in the two types of organisms. In Figure 4, the frequency of the "lethal" gene or nucleus is plotted against the number of generations of selection. The points on the curve of the disappearance of the recessive lethal gene in diploids are calculated from the simple formula: $q_n = q_o/(1 + nq_o)$ (DOBZHANSKY 1953), where q_o is the initial frequency of the lethal (0.34 in the present example), and n the generations of selection. The curve for *N. crassa* nuclear proportions is that of the PAN-1 line; q in this case represents the frequency of the *nic-2*, *al-2* nucleus, starting at 0.34.

It may be seen that the maintenance of recessive lethals in diploids is more efficient than maintenance of "lethal-bearing" nuclei in *N. crassa*. The level of the lethal nucleus in *N. crassa* drops below 0.01 between the tenth and fifteenth generations, whereas this level is reached by lethals in large diploid populations

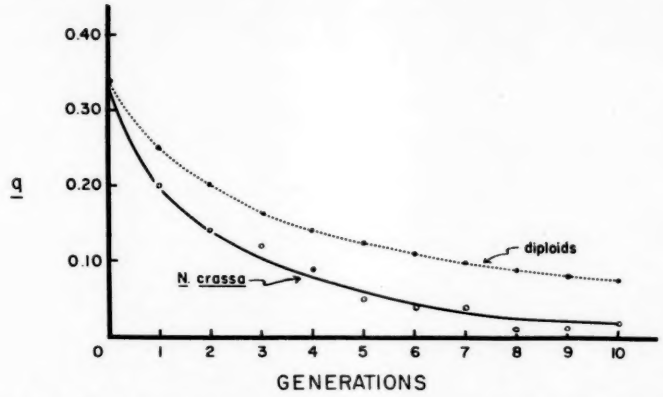


FIGURE 4.—Graphic representation of the decrease in frequency (q) of a "lethal" nucleus over ten asexual transfers of a heterocaryon of *N. crassa* or of a lethal gene during ten generations of random interbreeding of a diploid population.

only by the ninety-eighth if $q_o = 0.34$. The similarity in the pattern of change, however, is attested to by the general shape of the two curves.

SUMMARY

1. Conidial transfers of heterocaryons of biochemically deficient nuclear types of *N. crassa* have been made serially on media on which one of the homocaryotic conidial types could not propagate. The changes in the nuclear proportions over many generations have been observed, and compared to the changes expected on the basis of the conidial frequencies of the transfer inocula.

2. From known factors of the system, an algebraic model has been proposed to account for the observed changes in nuclear proportions during selection. This model includes a method for determining the selective values of conidial types under different nutritional conditions.

3. It has been found that nuclear types absolutely selected against may be maintained in a nuclear population for a considerable number of transfers by the association with another type in heterocaryotic conidia. This allows a heterocaryon to adapt quickly to changes of nutritional conditions.

4. The pattern of selection operating during the asexual reproduction of *N. crassa* heterocaryons has been compared to that operating during the sexual reproduction of diploids.

ACKNOWLEDGMENTS

I should like to thank DRS. R. P. LEVINE and J. R. RAPER for their interest and criticism during the course of this work, and DRS. R. P. LEVINE, J. R. RAPER,

and W. T. EBERSOLD for reviewing the manuscript of this paper. I should also like to thank the many others who gave helpful suggestions relating to this problem.

LITERATURE CITED

ATWOOD, K. C., and F. MUKAI, 1955 Nuclear distribution in conidia of Neurospora heterocaryons. Genetics **40**: 438–443.

BARRATT, R. W., D. NEWMEYER, D. D. PERKINS, and L. GARNJOBST, 1954 Map construction in *Neurospora crassa*. Advances in Genet. **4**: 1–93.

BEADLE, G. W., and V. L. COONRADT, 1944 Heterocaryosis in *Neurospora crassa*. Genetics **29**: 291–308.

DOBZHANSKY, TH., 1953 *Genetics and the Origin of Species*. Columbia University Press. New York.

DODGE, B. O., 1942 Heterocaryotic vigor in Neurospora. Bull. Torrey Botan. Club **69**: 75–91.

HANSEN, N. H., 1938 The dual phenomenon in the Fungi Imperfecti. Mycologia **30**: 442–455.

HANSEN, N. H., and B. E. SMITH, 1932 The mechanism of variation in the Fungi Imperfecti. Phytopathology **22**: 953–964.

HANSEN, N. H., and W. C. SNYDER, 1943 The dual phenomenon and sex in *Hypomyces solani f. cucurbitae*. Am. J. Botany **30**: 419–422.

HUEBSCHMAN, C., 1952 A method for varying the average number of nuclei in the conidia of *Neurospora crassa*. Mycologia **44**: 599–604.

KLEIN, D. T., 1958 Randomness of nuclear distribution in conidia of Neurospora heterocaryons. Z. Ind. Abst. Vererb. **89**: 323–327.

PITTENGER, T. H., and K. C. ATWOOD, 1956 Stability of nuclear proportions during the growth of Neurospora heterocaryons. Genetics **41**: 227–241.

PITTENGER, T. H., A. W. KIMBALL, and K. C. ATWOOD, 1955 Control of nuclear ratios in Neurospora heterocaryons. Am. J. Botany **42**: 954–958.

PROUT. T., C. HUEBSCHMAN, H. LEVENE, and F. J. RYAN, 1953 The proportions of nuclear types in Neurospora heterocaryons as determined by plating conidia. Genetics **38**: 518–529.

RYAN, F. J., 1950 Selected methods of Neurospora genetics. Methods in Med. Research **3**: 51–75.

RYAN, F. J., G. W. BEADLE, and E. L. TATUM, 1943 The tube method of measuring the growth rate of Neurospora. Am. J. Botany **30**: 784–799.

WESTERGAARD, M., and H. K. MITCHELL, 1947 Neurospora.V. A synthetic medium favoring sexual reproduction. Am. J. Botany **34**: 573–577.

THE SPECTRUM OF SEVERITY OF MUTANT EFFECTS. I. HAPLOID EFFECTS IN YEAST

ALLEN P. JAMES

Atomic Energy of Canada Limited, Chalk River, Ontario, Canada

Received April 29, 1959

IN estimating the genetic hazards of ionizing radiations to populations, it is important to know the frequency distribution of induced mutations in relation to the severity of their effects. Although it is well known that these effects vary from extreme to slight, most investigations have concentrated on large effect mutations; not only are these more easily detected, but they are likely to be of greater importance to an affected individual. However, mutations of small effect may be of considerable consequence to a population as a whole. And if their relative frequency is high, they may be of greater collective importance than are those whose effects are drastic.

There are few organisms where extreme technical difficulties do not preclude the detection of individual induced mutations of small effect. At present, studies of the frequency distribution of severities of induced mutations have been confined largely to Drosophila. Our knowledge of the effect of mutations in the homozygous state is derived mainly from the classic investigations of TIMOFEEFF-RESSOVSKY (1934), and of MULLER (1934) and KERKIS (1938). These showed that the ratio of detectable detrimental mutations to complete lethals is two or three to one. Because of technical difficulties, none of these studies provided reliable information on the severity distribution of nonlethal mutant effects. More recently, MULLER (1950) has presented indirect evidence that the distribution is bimodal, with one peak toward the lethal end and a second peak toward the normal end.

Yeast is singularly well adapted to an investigation involving the detection of mutant genes, and experimental techniques are available for studying much smaller individual mutant effects than can be detected even in Drosophila. This organism thus permits a study of the "normal" end of the distribution curve, about which we are at present almost completely ignorant. The organism can also be used to provide confirmation of the Drosophila results which now stand almost alone. The present report is concerned with the frequency distribution of X-ray-induced mutations as they affect the quantitative character of growth rate in yeast.

TECHNIQUES AND PROCEDURES

With *Saccharomyces cerevisiae*, mutants can be detected and their effects estimated without recourse to backcrossing, since the organism can reproduce in both the haploid and diploid phase. Furthermore, a single diploid cell normally

produces its meiotic segregants in the form of a group of four ascospores; if a simple genetic change has occurred, the segregants include two identical mutants and two "built-in" controls. Finally, repeated sampling can be accomplished through vegetative reproduction of a diploid cell prior to sporulation.

General procedure: The basic features of the experimental procedure are outlined in Figure 1. The descendants of a single inbred diploid cell were cultured through approximately 20 generations to the stationary phase. The cells were then transferred to buffer solution and divided into two cultures; one of these served as a control population and the other was irradiated. Both cultures were plated to yield 10–20 colonies per plate. A single-cell isolation was made from each colony as soon as these became clearly visible to the naked eye. Each cell was incubated for three days on a droplet of fortified agar medium at 21.5°C and the resultant clone was refrigerated in broth culture. These clones were sporulated and the haploid segregants of individual asci were used directly to estimate the genetic effects of irradiation.

Various phases of the experimental and analytical techniques will be described in detail in the following sections.

The character: The quantitative character, growth rate, was chosen for investigation in the expectation that it would be affected to some extent by a large proportion of all mutations. Further, in terms of selection pressures, rate of growth in yeast is analogous to the character, "ability to reach adulthood," commonly used in comparable investigations of Drosophila.

Special precautions are necessary if growth rate is to be a sensitive indicator of mutation. Because yeast is a unicellular organism, rate of growth can be described only in terms of population increase. Thus, although any clone may be

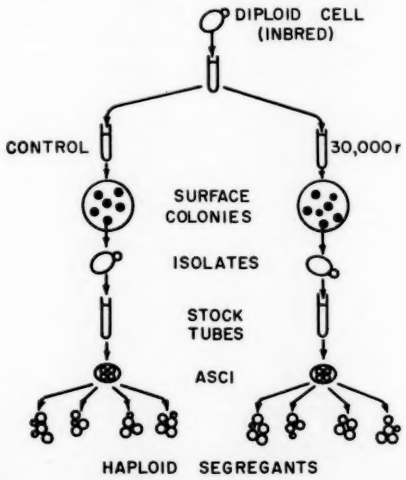


FIGURE 1.—General procedure for detecting mutants.

thought of as an "individual" for the purposes of this investigation, it would nevertheless respond as a population to selection pressures if there were internal mutations. As a result, spontaneous mutants might become predominant very quickly. Within a haploid culture such subsequent mutations can alter growth rates so as to obscure initial segregational differences; within a diploid culture they can lead to heterogeneity if not to complete replacement of the genotype.

With haploid segregants, the influence of spontaneous mutations was largely avoided by obtaining estimates of growth rate before such mutants had an opportunity to alter the population; the entire progeny of individual ascospores was used to estimate growth rate, and the collection of data was completed by the time an ascospore had produced no more than 20 generations of progeny. Genetic shifts within diploid cultures were kept to a minimum by permitting as little cell reproduction as was practicable in the interval between irradiation and sporulation.

Estimates of growth rate were calculated from the diameters of microcolonies derived from single ascopores. Fortuitously, the logarithm of the diameter of a microcolony when plotted against time, yields a curve that is essentially linear between diameters of 0.1 mm and 1.0 mm (10^3 and 10^5 cells). This fact permits the estimation of relative growth rate and obviates the determination of actual rates of cell division.

The procedure for growing haploid clones was designed to reduce environmental differences between any four segregants to a minimum. The four spores of an ascus were planted separately on a single large droplet of fortified agar medium on a coverslip. The whole was then inverted over a Van Tieghem cell, sealed, and incubated at 21.5°C. Dissections of asci were performed in daily "lots" of about 50. It was experimentally impossible to provide each test segregation with an assured nonmutant control segregation. However, the number of segregations from control and from irradiated clones was kept equal in each lot. Furthermore, except in rare instances, each clone was represented only once or twice in any lot. This procedure reduced the effect of lot differences (attributable to minor fluctuations in temperature of incubation) on the mean growth values of segregants from different diploid clones.

Details regarding medium and method of measuring microcolonies have already been published (JAMES and SPENCER 1958). Because of the large number of estimates required, colonies were measured only twice, at approximately 48 and 72 hours after planting of the spore.

Two partially independent values, each reflecting growth rate, were extracted from the data for each microcolony. One of these, the "one-point" value, was the average increase per hour in logarithm of diameter between time of planting of the ascospore and the final measurement. The other value, termed the "two-point" value, was based on the average increase per hour in logarithm of diameter during that interval when the plotted growth curve was essentially linear (i.e. when colony diameter was between 0.1 mm and 1.0 mm). With the latter value, experimental procedure frequently did not, in fact, permit both readings

to fall within this interval. Here a correction was made by adjusting the logarithm upward to the theoretical value expected for a completely linear curve.

Both growth values are, of course, influenced by other variables than the rate of cell division. The one-point value is affected by time of spore germination, and both values are affected by cell size and colony morphology. However, these factors are probably only of incidental importance, except with near-lethals, and here a large error does not affect the interpretation of experimental results. The one-point values must also be affected by a slight convexity of the growth curves which is found when logarithm of diameter is plotted against time. This results in an underestimate of actual differences, but the error must decrease with decreasing differences in colony diameters. The common origin of the cytoplasms of the four spores of an ascus may also lead to an underestimation of differences as determined by one-point values.

The strain of yeast: An inbred line of yeast, termed 4819 in this laboratory, was used. The origin of this strain will be described in detail because the method of inbreeding undoubtedly influenced its genotype which, in turn, should be considered in an interpretation of the experimental results.

Four methods of inbreeding were tried. One involved a search for mutants for sex within a suitable haploid line. It was intended that a mutant and nonmutant be mated to produce a diploid strain homozygous at all loci except sex. The second method involved a search for a mutant for sex within a self-diploidized line. Such a mutant could be detected by its ability to sporulate. Either method would yield an "unselected" homozygote but neither was successful. Recourse was then made to controlled "brother-sister" matings of selected segregants. Numerous attempts to obtain an inbred line ended either in morphologically unsuitable strains or in serious lessening of the ability to sporulate. A satisfactory inbred line was finally obtained by a technique of uncontrolled "brother-sister" matings in which the intensity of inbreeding was reduced. Intact asci were isolated and incubated. Following selection of a diploid colony with good morphological features and sporulating ability, the process was repeated. Interestingly, in early generations the colonies were either uniformly haploid or diploid in appearance, whereas in later generations most of the colonies were obviously sectored for haploid and diploid cells. The inception of equal growth rates of haploids and diploids provides an indication of the point at which heterosis had been eliminated.

At the end of 14 generations of inbreeding it was evident that the strain had become unsuitable for growth rate studies; it produced respiratory-deficient cells with an exceptionally high frequency. A detailed analysis of this behavior has been published (JAMES and SPENCER 1958). Stability was introduced into the strain in the following manner: haploid segregants of the fourteenth generation were plated and a stable variant colony detected by its smooth contours. The variant was mated to a nonmutant segregant of opposite sex. The resultant diploid was itself stable, but segregated 2:2 for stability, facts which indicated the presence of a dominant mutant gene. Stable segregants of this diploid were

mated to produce a sixteenth generation. Inbreeding was then continued in the usual manner for a further ten generations without the reappearance of instability. The final strain, 4819, having been inbred for 26 generations, was a fast growing diploid with satisfactory colony morphology and good sporulating ability.

Compilation and statistical analysis of data

Recessive lethals and near-lethals: Recessive lethals and near-lethals in yeast are easily detected since two of the ascospores from each heterozygote will not produce normal colonies. In many instances spore germination itself is not affected by the presence of a recessive lethal; lethal-bearing spores may germinate to produce at least one bud or even a minute microcolony. Such spores have been termed abortive and their initial growth can best be attributed to the derivation of their cytoplasms from the heterozygote. As one might expect, the line between lethal and near-lethal is easily shifted with some highly detrimental mutations; segregations from a single clone sometimes indicate lethality, at other times near-lethality (see Table 2). The extensive testing required to demonstrate that a mutation was in fact lethal and not near-lethal was considered to be unwarranted, and a clone was classified as near lethal if any pair of affected spore colonies grew in the interval between 48 and 72 hours after planting.

A single segregation of two normals and two lethals or near-lethals is a strong indication that a test clone is mutant, because such segregations in supposedly mutant-free cultures are rare (See Table 3). In practice, at least one more dissection was made to confirm the genotype. If none of these yielded more than two "normal" spores, the clone under test was classified as mutant for a lethal or near-lethal.

On occasion, recessive lethality is expressed by the complete inability of spores to germinate. Because this characteristic may also be attributed to damage at dissection, positive identification of such lethals requires repeated dissections. As germination of two or fewer spores occurred only eight percent of the time in presumed lethal-free clones, four dissections were considered sufficient for this purpose.

Nonlethal mutations: Only data from those dissections which yielded four spore colonies were used in the detection of nonlethal mutations. Mutants were identified through analyses of variance; the pooled variance of individual tetrad growth values from a test clone was compared with that of an assumed nonmutant (base control) clone. It should be noted that this method does not take advantage of the paired nature of the data.

To insure unbiased sampling, it was originally planned that each clone be represented by five segregations, excepting those instances where mutation was obvious. However, not infrequently, an individual segregation was clearly unrepresentative of the clone from which it was sampled. When this discrepancy was extreme, the mean square showed a significant departure from homogeneity and the data could be discarded. This procedure unfortunately reduced the data available for detecting mutants in some clones. An effort was therefore made to

identify as many as possible of these unrepresentative segregations prior to the analysis and to repeat the sampling, without in any way biasing the data. Discarded data are listed in Table 3. The measures taken to disclose them were as follows:

(a) Data from a segregation were discarded when there was evidence of an extreme delay in germination of one or more ascospores. Although time of germination was, in general, synchronized at low incubation temperatures, it was sometimes evident that a delay had occurred. These delays directly affect the "one-point" growth values and increase the experimental error of the "two-point" growth values. Colonies were inspected microscopically at 24 hours, and data from a segregation were discarded if any germinating spore had produced six or fewer cells.

(b) Data were discarded when cells of all four segregants from an ascus had the oval appearance of diploids, an observation suggesting that they had originated from a tetraploid.

(c) Irregular segregations in which one of the four segregants was either an abortive or an excessively fast-growing variant were discarded. The latter were easily recognized; their diameters were relatively large (in excess of 0.3 mm at 48 hours) and their colonial outlines very smooth. It is of interest that they were frequently accompanied by excessively slow-growing segregants (see Table 3). A third type of irregular segregation, one involving the appearance of respiratory-deficient segregants, was known to occur but was not eliminated prior to analysis.

(d) Exceptional segregations suggesting genetic heterogeneity within a clone were discarded when heterogeneity for lethals and near-lethals or for fast-growing variants of the type described above were involved.

When the effect of mutation was extreme its direction (i.e. positive or negative) was obvious. The direction was less apparent for smaller effects because daily fluctuations in temperature of incubation, although minor, created significant differences between mean growth values for lots. A discrimination was achieved by the use of presumed mutant-free clones. These constituted all clones in which the mean growth values of the two slower and the two faster segregants differed, on the average, as little or less than did the two corresponding segregant pairs in the base control (2.3 percent). Data obtained with these clones were now used to calculate an over-all mean growth value of 29.4 and an adjustment for each lot. These lot adjustments were then applied to individual segregant values of all test clones. The over-all mean growth value for segregants of each test clone was now compared with the value 29.4. The direction of variation was determined by the sign of the difference. An error in the estimated mean growth value for non-mutant segregants would, of course, affect the relative numbers of positive and negative test clones in those clones where mutation was not detected. However, the estimated extent of a mutant effect, whether or not it is demonstrated statistically, will not be affected by any error in this value.

RESULTS

Survival of diploid cells after exposure to 30,000 roentgens was 46 percent, as measured by formation of visible colonies. Thirty-four unselected control colonies and 100 unselected colonies from plates of irradiated cells were picked and the clones derived from them were tested for genotype. Surprisingly, statistical analysis showed that the one-point growth values were a more sensitive measure of genotypic differences than were the two-point values, and unless otherwise stated the former are used in the presentation of results in this report.

Gross results are presented in Table 1. Details of the different genetic effects will be treated separately.

Sporulation

Of the 134 test clones, 16 from irradiated cells could not be induced to sporulate. Spectroscopic analysis (by the method of RAUT 1953) indicated that seven of these were respiratory deficient. The data thus indicate an induced frequency of seven percent for respiratory deficiency and of ten percent for other changes involving an inability to sporulate. These clones were not further tested.

Gross chromosomal aberrations

In yeast, reciprocal translocations would lead to the production of equal numbers of asci from which none or all of the segregants are viable. Asci containing two viable and two inviable spores would also be expected, their frequency depending on the distance between centromere and points of breakage.

There was evidence that few if any of the lethal-free clones, whether control or irradiated, contained reciprocal translocations. Although many of these clones were insufficiently tested to unquestionably disprove the presence of a translocation, complete segregant failure was found in only two of 34 control clones and in five of 62 irradiated clones free of lethals or near-lethals. Here the failures occurred as isolated instances and the highest frequency was one in seven asci. It is quite possible that some of the clones classified as heterozygous for lethals or

TABLE 1

Mutants detected among control and irradiated cells of a yeast population

Type	Control	Irradiated	Percent estimated induction frequency (30,000r)
Respiratory-Deficient	0	7	7
Nonsporulating	0	9	10
Lethal	0	13	17
Near-Lethal	1	9	52
Mutant for Growth	8	31	
Nonmutant	25	31	
Total Cells Tested	34	100	

TABLE 2

Segregations of clones classified as heterozygous for recessive lethals or near-lethals

Clone no.		Clone no.		Clone no.		Clone no.	
C–22	++A⁺A⁺	I–23	++−−	I–56	++A⁺A⁺	I–83	++A⁺A⁺
	++A⁺A⁺		++−−		++A A		++A A
	++−A⁺		++−−		++−−		++A A
			+−−−				
I–2	++A⁺A⁺	I–29	++A A	I–59	++A A	I–93	++−A⁺
	+−A⁺A⁺		+−−−		++−A		++−A
							++−−
				I–63	++A A		++−−
I–6	++A⁺A⁺	I–30	++A⁺A⁺		++−−		
	++A⁺A⁺		++A⁺A⁺		++−−		
			++A⁺A⁺		++−−	I–54	++A⁺A⁺
							+−−A
			++A A	I–66	++A A		+A A A
I–7	++A A		++A A		++−−		
	++A A		++A A		+−−−		
	++A A		−−−A			I–60	+A A A
	++−A			I–69	++A⁺A⁺		+−−A
		I–52	++A⁺A⁺		++A⁺A⁺		−A A A
I–18	++A⁺A⁺		++A A		++A A		
	++A⁺A⁺		−−A A				+−−A
	++A A						+−−−
	++A A			I–76	++−−		+−−−
	++A A	I–53	++A A		++−−		−−−−
	++A A		++−−		++−−		
	+−A A		++++*		++−−	I–89	+A A A
	−−A A						+A A A
	−−A A						+A A A
	−−−−						−−A A

* Cells of spore colonies appeared diploid.
\+ Spore produced a "normal" colony.
A⁺ Spore germinated to produce a minute but growing microcolony.
A Spore germinated to produce one bud or a stationary microcolony.
— Spore did not germinate.

near-lethals were actually translocation clones. However, the fact that all clones segregated "lethals" either consistently or else only rarely (see Tables 2 and 3) suggests that translocation clones were not frequent in this class.

Recessive lethals and near-lethals

Of the 34 control clones, one was classified as heterozygous for a near-lethal. Of the 84 sporulating clones derived from irradiated cells, 13 were classified as heterozygous for lethals and nine as heterozygous for near-lethals. Segregations of these clones are presented in Table 2. There can be little doubt of the high accuracy of the lethal-near-lethal classification. The subclassification of the clones into lethal or near-lethal is no doubt less accurate (see previous discussion), but certainly there is a cluster of near-lethals.

TABLE 3

Statistical analyses of clones free of lethals and near-lethals

*1	2	3	4	5	6	7	8	9	10
Base control	C–5	100	10	9	4 (+ + + A) 1 (+ + A A) 1 (+ + + + − −)	11	64	.977	
Control	C–1	9	3	0	1 (+ + A A)	0	5	1.024	
	–2	8	2	1	0	3	2	.985	
	–3	6	1	0	0	1	4	1.019	
	–4	5	2	1	0	0	2	.863	**
	–5	7	2	0	0	0	5	.982	
	–6	7	1	1	0	1	4	1.013	
	–7	12	2	1	3 (+ + + + + +) 1 (+ + + A)	0	5	.982	
	–8	5	0	0	0	0	5	1.111	**
	–9	12	4	3	0	0	5	.974	
	–10	3	0	1	0	0	2	.868	**
	–11	8	1	2	0	0	5	.949	**
	–12	8	3	0	0	0	5	.973	
	–13	7	1	0	1 tetraploid	0	5	.959	**
	–14	6	0	1	0	0	5	1.027	
	–15	7	1	1	0	0	5	.968	
	–16	5	0	0	0	1	4	.976	
	–17	8	1	1	0	2	3	.981	
	–18	6	1	0	0	1	4	.980	
	–19	10	3	1	1 (+ + + + + +)	0	5	1.023	
	–20	12	5	2	0	2	3	1.020	
	–21	9	2	1	1 (+ + + + − −)	0	5	.979	
	–23	8	2	1	0	0	5	1.092	**
	–24	10	4	1	0	0	5	1.017	
	–25	7	1	1	0	0	5	.914	**
	–26	9	2	2	0	0	5	1.018	
	–27	7	4	0	0	0	3	.899	**
	–28	6	0	0	1 (+ + + A)	0	5	.972	
	–29	5	0	0	0	0	5	1.020	
	–30	6	0	1	0	0	5	.971	
	–31	9	2	1	1 (+ + + A)	1	4	.981	
	–32	6	0	1	0	1	4	.976	
	–33	12	5	2	0	3	2	.984	
	–34	10	3	2	0	0	5	.972	
Irrad.	I–1	9	3	1	0	3	2	.991	
	–3	7	1	1	0	0	5	1.030	
	–4	6	0	0	1 (+ + + + −)	0	5	.975	
	–5	7	0	0	1 tetraploid 1 (+ + + + − −)	1	4	.987	
	–8	9	4	0	0	0	5	.806 1.103	**
	–9	2	0	0	0	0	2	.387	**
	–11	6	1	0	0	0	5	.965	**
	–12	6	0	1	0	0	5	.977	

TABLE 3—(Continued)

Statistical analyses of clones free of lethals and near-lethals

*1	2	3	4	5	6	7	8	9	10
	−13	12	6	0	1 tetraploid	0	1	1.094	**
					1 (++ ++ A A)				
					1 (++ ++ + A)				
					1 (++ + A A)				
					1 (++ + + A)				
	−14	15	5	5	0	0	5	.979	
	−15	5	0	0	0	0	5	.965	**
	−17	10	4	1	0	1	4	.981	
	−19	10	4	0	1 (++ + + −)	1	4	.968	
	−20	5	0	0	0	2	3	.982	
	−21	7	0	1	1 (++ ++ − −)	0	5	.979	
	−22	7	2	0	0	0	5	.983	
	−24	3	0	0	0	0	3	.899	**
	−25	9	3	0	1 (++ + + −)	0	5	.965	**
	−26	11	4	0	2 (+ + + A)	0	5	.962	**
	−28	7	2	0	0	3	2	1.012	
	−31	5	2	2	0	0	1	.690	**
	−33	6	1	0	0	0	5	1.017	
	−34	7	2	0	0	0	5	.973	
	−35	8	1	2	0	2	3	1.009	
	−36	11	5	1	0	1	4	1.024	
	−38	5	0	0	0	0	5	.978	
	−40	8	2	0	1 (+ + + A)	0	5	.956	**
	−41	7	0	2	0	0	5	1.181	**
	−42	10	4	1	1 (++ + + −)	0	4	.928	**
	−43	4	3	1	0	0	2	.880	**
	−44	11	4	2	0	0	5	.953	**
	−45	7	1	0	1 (++ − − −)	0	5	.955	**
	−46	8	1	2	1 tetraploid	0	4	.873	**
	−47	9	1	0	2 (+ + A A)	0	5	.965	**
					1 (+ + + A)				
	−48	6	2	1	0	0	3	.781	**
	−49	10	5	0	0	0	5	.838	
								1.089	**
	−50	7	1	1	0	0	5	.964	**
	−51	2	0	0	0	0	2	.796	**
	−55	6	0	1	0	0	5	.959	**
	−57	6	1	0	0	0	5	.976	
	−58	3	0	0	0	0	3	.890	**
	−62	11	4	1	1 (+ + A A)	1	4	1.016	
	−65	6	0	0	1 (++ + + −)	1	4	.972	
	−67	13	6	2	0	0	5	1.135	**
								.797	
	−68	10	3	2	0	1	4	.966	
	−71	8	1	1	1 (++ ++ + +)	1	4	1.027	
	−72	8	3	0	0	0	5	.972	
	−74	9	2	2	0	0	5	.970	
	−75	8	2	0	1 (+ + A A)	0	5	1.016	

–78	2	0	0	0	0	2	.798	**
–79	8	0	3	0	0	5	.952	**
–80	9	1	3	0	0	5	.554	**
–81	10	4	0	1 (+ + A A)	0	5	.977	
–82	10	5	0	0	2	3	.994	
–84	8	3	0	0	0	5	.822	**
–86	6	1	0	0	1	4	.983	
–87	11	5	0	1 (+ + + + + +)	1	4	1.024	
–88	10	3	1	1 (+ + + A)	1	4	.972	
–91	9	7	0	0	0	2	.943	**
–92	3	0	0	0	0	3	.765	**
–94	11	4	1	2 (+ + + – –)	0	3	.871	**
				1 (+ + + A)				
–98	11	4	2	0	3	2	1.018	

* Meanings of numbers used as column headings: 1—Source. 2—clone number. 3—No. asci dissected.
4—No. asci in which germination of spores was incomplete.
5—No. asci in which germination of one or more spores was delayed.
6—Exceptional segregations: +, "normal" colony; + +, excessively fast; —, excessively slow; A, abortive.
7—No. segregations eliminated from data as statistically nonhomogeneous.
8—No. segregations used in detection of mutation.
9—Phenotypic effect. This is the mean clonal value for the sum of two more variant segregant growth values divided by the sum of the two less variant growth values. The term is a measure of the fractional increase, or decrease, in rate of growth of the more variant segregants of a clone. For example, if the phenotypic effect is .850, then the rate of cell division of the two slower segregants has been estimated to be, on the average, 85 percent that of the two faster (and presumably nonmutant) segregants. Effects for I–8 and I–49 were calculated from appropriate segregant values. I–67 produced only one nonmutant segregant; here estimates were obtained by using values derived from presumed mutant-free clones. It happened that all clones having effects lower than .965 or higher than 1.036 were classified as probable mutants in statistical tests.
10—Of statistical significance. ** indicates significance of the variance ratio at the 0.01 level.

Clones containing lethals or near-lethals fell into two categories: (1) those with segregations best explained by the presence of a single detrimental or at least not at variance with this interpretation, and (2) those with segregations indicating a more complex genetic change. The four clones of column 4, Table 2, fall into this latter category. It is possible that inversion or dominance of a gene affecting germination is responsible for this complex behavior but it is not necessary to invoke such an interpretation. The frequency of clones with lethals or near-lethals is so high as to lead to the expectation that several clones contain two or more of independent origin. It seems likely that the instances of complex lethality are of this nature. An X-ray-induced frequency of 17 percent for lethals is indicated.

Nonlethal mutations

Growth-rate data from all clones that did not contain lethals or near-lethals were submitted to statistical analysis. Summarized results are presented in Table 3. Probable mutants were found in eight of 33 control clones and in 31 of 62 "irradiated" clones. The five percent level of significance was chosen as a criterion for classifying clones as probable mutants, but in fact all significant variance ratios reached the one percent significance level, so that the chance of overestimating the number of mutants is considered to be slight.

Positive mutations: There was little evidence that any simple positive mutations were induced by X-rays. The frequency of positive mutations among control colonies was two in 33; among irradiated colonies it was five in 62. In each of

these instances, the mutant effect was so extreme as to be clearly evident from a visual examination of the segregant colonies.

The two control clones, C-8 and C-23, segregated as simple monohybrids for positive mutations. The five irradiated clones, on the other hand, showed greater complexity of the diploid genotype. Only one, I-41, segregated as a simple monohybrid. Complete and incomplete segregations from the remaining four clones indicated the following genetic constitutions: I-8 and I-49 behaved consistently as though heterozygous for both a positive and a negative mutation in which the negative mutation was epistatic to the positive. I-13 may have had an inversion; it produced abortive spores in four of five segregations. Otherwise it segregated as a monohybrid for fast growth. I-67 was more difficult to characterize; in four of five segregations it produced two obviously positive and two obviously negative mutant spores. The remaining segregation produced only one obviously positive mutant spore but, again, two negative. Repulsion linkage of positive and negative mutant genes is a possible explanation of the behavior of this clone.

Nonlethal negative mutations: Negative mutations other than near-lethal were detected in six out of 33 control clones and in 28 out of 62 irradiated clones.

The frequency distribution of the effects of the various mutations is shown in Table 4. Here all test clones have been arranged by their "phenotypic" effects. It is evident that the distribution of mutant effects is bimodal; there is a peak at the near-lethal end and a second peak at the near-normal end.

Further, it is apparent that the distribution in the regions adjacent to the normal is greatly skewed; there is a preponderance of negative over positive effects. This is best shown in Figure 2, where the frequency distribution at the normal end is presented in expanded form. It seems likely that few if any undetected positive mutations were induced.

DISCUSSION

There can be no doubt that the techniques used here were successful in permitting the detection of mutations of both large and small effect. However, in such an investigation as this, biases in the data, even if small, can seriously affect the interpretation of results. Consequently, it is pertinent to consider the extent

TABLE 4

The frequency distribution of phenotypic effects in clones derived from control and irradiated cells

	Lethals	Near-lethals						Negative mutants										Positive mutants			Total
Phenotypic effect	0	.055	.125	.195	.265	.335	.405	.475	.545	.615	.685	.755	.825	.895	.965	1.0	1.035	1.105	1.175	1.245	
Control	0	0	1	0	0	0	0	0	0	0	0	0	0	2	4	16	9	1	1	0	34
Irrad.	13	0	1	4	4	0	1	0	0	1	0	1	6	5	14	21	10	3	0	1	85*

* I–8 and I–49, being obviously heterozygous at two loci, are each represented twice. I–67 is unrepresented.

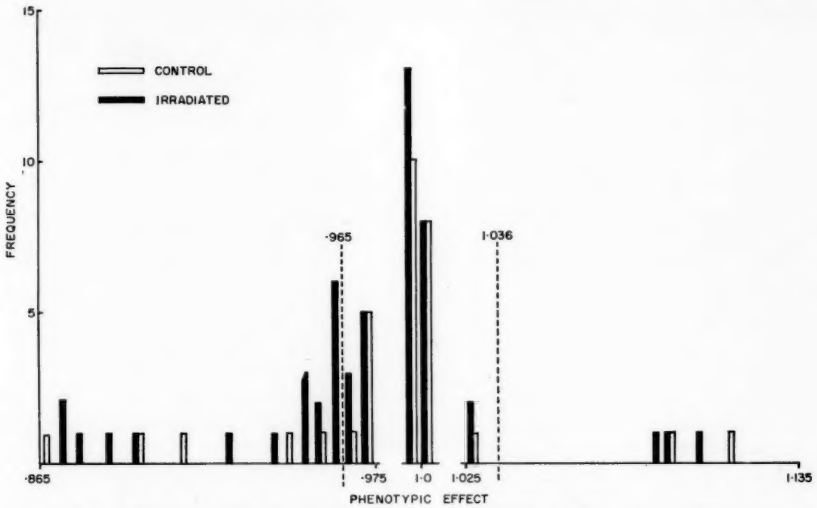


FIGURE 2.—The frequency distribution, by phenotypic effect, of near-normal clones from individual control and irradiated cells. The discontinuity between 0.975 and 1.025 is necessitated by the expected rarity of values in the region of 1.0.

and direction of any biases in the information with respect to (a) clones tested and (b) population sampled.

(a) As to the test clones themselves, it is most unlikely that any classed as mutant for small effects were in fact nonmutant. But clones classed as mutant for larger effects were less thoroughly sampled and additional dissections might have disclosed that some of these were heterogeneous in genotype. Again, it is entirely possible that some mutant clones passed undetected. If so, most of these must have been small-effect mutants: (1) Further sampling of individual clones might well have extracted additional minor mutations but could hardly have uncovered any additional major mutations. (2) Minor mutations would not have been observed in dihybrids for minor and major mutation because of the nature of the statistical treatment. (3) Adjustments for heterogeneity of variances within clones, in retaining more uniform data and eliminating more exceptional data, almost certainly resulted in the classification of some mutants as nonmutants. Here also the frequency of minor mutations would be more greatly underestimated than the frequency of major mutations. One may suspect that the procedure also resulted in the classification of dihybrids for two minor mutations as nonmutants.

Concerning the estimates of mutant effect, the distribution of these is biased by an underestimation of the mutant effect in slow growing segregants as mentioned previously. But the two-point values, corrected for curvilinearity of the plotted growth curves, provide a measure of the extent of this bias. In Figure 3 the phenotypic effects as derived from each set of values have been plotted against

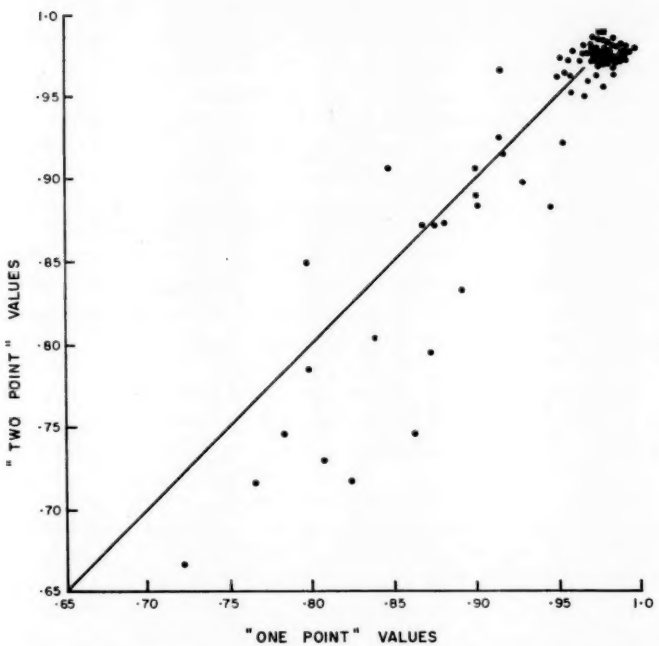


FIGURE 3.—Correlation of phenotypic effects in test clones as obtained from two partially independent sets of growth values.

each other for effects between 0.65 and 1.0. It is evident that the bias is negligible in the near-normal range.

(b) As to whether the clones themselves were representative of the sampled populations of cells, the most important factor to be considered is that of spontaneous mutations. These may occur either before or after irradiation. Those which occur prior to irradiation are detected in the control and are thus of little consequence unless their frequency is exceptionally high. Those which occur after irradiation can be of greater importance because they are imposed on two populations which now differ in genetic constitution. However, even here they are of consequence only when they result in increased growth rate in diploids already suffering from a reduced rate of growth. Where these occur as true back mutations they reduce the mutation frequency as estimated. Where they occur at a different locus the resulting dihybrids are probably classified as such (I-13 and I-62 may serve as examples) or, if both mutant effects were minor, as non-mutants.

Induced somatic crossing over and delayed mutation could both result in clonal heterogeneity and a reduction in estimated mutant frequency. It is, however,

unlikely that either of these phenomena occurs with sufficient frequency to affect the data seriously.

Altogether, it seems reasonable to infer that the distribution of mutant effects is representative of the actual distribution but that the induced frequency of minor mutations is probably underestimated and the frequency of major mutations is, if anything, slightly overestimated.

These results completely corroborate the Drosophila evidence that the distribution of induced mutations is bimodal with respect to severity of effect. Further, the ratio of the frequency of induced nonlethal to lethal mutations, which must certainly be in excess of 3:1, is in approximate agreement with the Drosophila data.

The experimental procedure permitted a study of the distribution of effects in a region previously unexplored in that it enabled detection of individual mutations which affect rate of growth as little as 3.5 percent. It is apparent that the frequency of deleterious mutations continues to increase as phenotypic effect decreases, and in the most common class the depressing effect of individual mutations is 3.5 percent or less.

Perhaps the most interesting aspect of this study is to be found in its indication that positive mutations of small effect were induced with a low frequency if at all. Of 14 mutations having an effect of less than six percent, all were negative. It is, of course, generally accepted that the frequency of positive mutations, whether spontaneous or induced, is lower than that of negative mutations. This opinion is based partly on *a priori* reasoning and partly on results of research in the agricultural uses of mutations. There is, however, still considerable discussion as to how often, in fact, positive mutations can be expected on the basis of the Drosophila curve (see NEEL and SCHULL 1956). Under these circumstances it is worth considering whether the apparent absence of X-ray-induced positive mutations in this study can be regarded as a general phenomenon or merely as a property of the strain and character used.

Certainly positive mutations for rate of growth would be rare in a strain long subjected to a constant environment. But growth rate in strain 4819 was obviously not maximal even though there was selection for an increased rate of growth during the inbreeding program that produced it; the introduction of stability in the fourteenth generation almost certainly resulted in a reduction in growth rate, and the presence of excessively fast growing variants in both control and irradiated populations attested to the ability of the strain to undergo positive mutation.

The absence of small positive mutations in strain 4819 might be attributed to the presence of a single genetic block in a genotype otherwise constituted for a faster growth rate. Such a situation would necessitate the occurrence of a specific mutation before other and lesser positive mutations could be effective. However, under such a hypothesis one would expect small potentially negative mutations to be equally ineffective in the strain, and this was not the case. Furthermore it

might reasonably be expected that the effectiveness of minor mutations in any strain is dependent on prior mutation at one or a few loci. Under these circumstances strain 4819 can hardly be considered exceptional. It therefore seems likely that the strain of yeast under study here is not exceptional and that X-ray-induced small-effect positive mutations are rare whereas small-effect negative mutations are abundant.

SUMMARY

The spectrum of X-ray-induced mutations, as they affect the quantitative character growth rate in haploid yeast was studied. Mutations with effects ranging from lethality to an estimated 18 percent increase in rate of growth were detected. The smallest detected effect was estimated at 3.5 percent. The distribution of induced mutant effects was bimodal, with a peak at the lethal and a second peak at the normal end. The ratio of induced nonlethal to lethal mutations was in excess of 3:1. The distribution of effects was massively skewed toward the normal end. No evidence of X-ray-induced small-effect positive mutation was found, although mutations of large positive effect were present in both control and irradiated populations.

ACKNOWLEDGMENTS

The author acknowledges the technical assistance of MR. P. E. SPENCER and is indebted to MISS P. M. CLARKE for advice on the statistical analysis of the data.

LITERATURE CITED

JAMES, A. P., and P. E. SPENCER, 1958 The process of spontaneous extranuclear mutation in yeast. Genetics **43**: 317–331.

KERKIS, J. J., 1938 The frequency of mutations affecting viability. Izvest. Akad. Nauk S.S.S.R. (Otdel. Mat.-estestven., Ser. Biol) 75–96.

MULLER, H. J., 1934 Radiation-genetics. (Abstr.) Verhandl. 4th Intern. Kongr. Radiol. (Zurich) **2**: 100–102.

1950 Our load of mutations. Am. J. Human Genet. **2**: 111–176.

NEEL, J. V., and W. J. SCHULL, 1956 The effect of exposure to the atomic bombs on pregnancy termination in Hiroshima and Nagasaki. Natl. Acad. Sci.-Natl. Research Council, Washington. Publ. No. **461**.

RAUT, C., 1953 A cytochrome deficient mutant of *Saccharomyces cerevisiae*. Exptl. Cell Research **4**: 295–305.

TIMOFEEFF-RESSOVSKY, N. W., 1935 Auslosung von Vitalitatsmutationen durch Rontgenbestrahlung bei. *Drosophila melanogaster*. Nachr. Ges. Wiss. Gottingen. (Math.-Physik. Kl., Fachgruppen VI. Biol.) **1**: 163–180.

GENOTYPIC-ENVIRONMENTAL INTERACTIONS FOR VARIOUS TEMPERATURES IN DROSOPHILA MELANOGASTER

P. A. PARSONS[1]

University of California, Davis, California

Received May 6, 1959

INTERACTIONS between genotype and environment are defined as the differing behavior of similar genotypes in diverse environments. Evidence is accumulating that such interactions are common (LERNER 1958; MATHER 1955.) The mechanism by which populations react to different environments is difficult to assess; however, by definition, populations of genotypes exhibiting greater stability or homeostasis than others are less liable to be affected by fortuitous fluctuations of the environment. Populations exhibiting a high degree of stability or homeostasis may be expected, therefore, to show genotype-environmental interactions of less importance than populations with low stability.

In a population with low stability, the genotypic variance obtained in one environment may be radically different from that obtained in another, whereas in a population with high stability there should be a greater correlation between various environments. A critical factor in a breeding program is the magnitude of the genotypic variance of a population over the multiplicity of environments likely to be encountered.

In this paper, data are presented on the ability of *D. melanogaster* larvae to emerge as adults under six different temperature regimes. Three inbred lines, their hybrids and the F_2 generation are considered in an attempt to study the relationships between homozygosity, homeostasis or stability, and the magnitude of genotype-environmental interactions. In a similar, but considerably smaller experiment, THODAY (1953) studied the mean number of adults emerging from cultures of ten eggs for two inbred lines and their hybrids under four temperature regimes. He noted that the F_1 flies survived extreme temperatures better than the parents and therefore had greater stability for the specific environments involved. He did not, however, carry his analysis further than this.

MATERIALS AND METHOD

The three inbred lines were:

Oregon (OR)—sib-mated for 260 generations.
Sacramento (SAC)—sib-mated for 172 generations.
Bikini (BIK)—mass inbred since 1947.

The six possible F_1 hybrids and F_2's are included in the analysis.

Before commencing the main experiment, hatchability estimates were made from 20 samples of 150 eggs for each inbred line, F_1 and F_2. From this study, the

[1] Present address: Department of Genetics, University of Cambridge, Cambridge, England.

number of eggs expected to give 150 larvae was calculated, hence providing a tolerably accurate method of ensuring approximately 150 larvae per replicate in the main experiment. Each replicate in the main experiment consisted of a vial with about ten gms of food in which the number of eggs expected to produce 150 larvae was placed. Ten replicates of each inbred line, F_1, and F_2 were set up for each of the following six temperature regimes:

15°C
24°C for eight hours and 15°C for 16 hours (24°/15°C)
24°C
24°C for eight hours and 31°C for 16 hours (24°/31°C)
31°C
31°C for 16 hours and 15°C for eight hours (15°/31°C).

The flies at 15°C were kept in a refrigerator, those at 24° in a constant temperature room and those at 31°C in a waterbath. At 10 A.M. and 6 P.M., the replicates under the fluctuating temperatures were changed from one temperature to the other, so that the 16 hour period represents the night and the eight hour period the day.

At 15°C flies take 20 days to emerge on the average, compared with ten days at 24°C, and eight to nine days at 31°C. The temperature 31°C was chosen as it is partly or wholly lethal to some strains.

The adult flies were scored every two days until emergence was complete. The number of adult flies emerging from 150 larvae provides a measure of the combined effects of larval and pupal mortality. In THODAY's (1953) experiment, no correction for differential hatchability was made. His emergence figures therefore depended on hatchability as well as larval and pupal mortality.

Hatchability estimates: The hatchability estimates are presented in Table 1. The hatchability of the OR and SAC inbred lines is low compared with BIK. The two techniques of inbreeding, sib-mating and mass inbreeding, may explain this difference. Under sib-mating, randomly selected single pair matings form each generation, whereas under mass inbreeding, flies are transferred in mass from generation to generation, so that competition is more severe and favorable genotypes, which are probably heterozygotes, may unconsciously be selected more readily than under sib-mating. The F_1 figures are, with minor exceptions, similar

TABLE 1

Hatchability estimates—mean number of hatched eggs from 20 replicates of 150 eggs with standard errors

OR	134.3 ± 4.5	OR × SAC	127.6 ± 5.9	OR × BIK	141.7 ± 3.6
SAC	119.8 ± 4.5	SAC × OR	117.6 ± 7.6	BIK × SAC	142.2 ± 3.2
BIK	144.7 ± 2.8	BIK × OR	141.1 ± 3.3	SAC × BIK	132.8 ± 7.0
			F_2's		
	OR × SAC	147.7 ± 2.4	OR × BIK	147.8 ± 1.1	
	SAC × OR	146.8 ± 2.2	BIK × SAC	139.3 ± 3.7	
	BIK × OR	147.1 ± 2.0	SAC × BIK	147.1 ± 2.5	

in magnitude to the inbred lines. The crosses with BIK as the maternal parent give values similar to the BIK inbred line.

The maternal parent appears to determine hatchability of the eggs, hence the F_1's and inbred lines hatched from eggs of the same maternal genotype give similar hatchability estimates. A similar result was obtained by CLOUGH and COCK (1957) in chickens.

Similarly, the standard errors of the hatchability estimates are equivalent in magnitude between the inbred lines and F_1's. The standard error of the BIK inbred line and the crosses with BIK as the maternal parent are smaller, on the whole, than the standard errors of the other inbred lines and crosses. This agrees with the interpretation that the BIK inbred line is perhaps more heterozygous than the SAC and OR inbred lines. The lesser variation of the BIK inbred line is an expression of its stability. The postulated heterozygosity of the BIK inbred line therefore gives it a superior homeostatic function compared with the SAC or OR inbred lines.

The F_2 hatchability figures represent the ability of the F_1 eggs to hatch. These hatchability figures, with the exception of the mating BIK × SAC, are higher than those for the corresponding inbred lines and F_1's. On the whole, therefore, these hatchability figures demonstrate heterosis. Furthermore, the F_2's have lower standard errors than the inbred lines and F_1's, thus exhibiting a superior homeostatic function compared with the F_1's and inbred lines. As hatchability appears to depend on maternal parent, these figures are therefore in agreement with recent theories on the relationship of heterozygosity and homeostasis.

Emergence of adults: In Table 2 the results for the emergence of adult flies are presented. Each entry represents the mean of ten replicates with its standard error. Marginal means are given for each temperature, inbred line, F_1, and F_2 with their standard errors. A few observations may be made directly on this table without further calculation.

At 24°C, the temperature at which the stocks were kept prior to the experiment, the highest proportion of larvae became adults. The next most favorable temperatures were 15°C and 24°/15°C. It is perhaps somewhat surprising that there is so little difference between the results for 15°C and 24°/15°C as a period at 24°C might have been expected to increase the emergence. Frequencies of emergence of adults from cultures kept at the three temperatures which included 31°C were not as high as the frequencies observed with cultures kept at the other temperatures. As might be expected, the lowest emergence was obtained at 31°C. At this temperature, the inbred lines OR and SAC had very poor emergence (an average of eight and 17 respectively). A period of eight hours daily at 24°C or 15°C and the remaining 16 hours at 31°C, was sufficient to increase the OR and SAC emergence two to eight times when compared with continuous exposure to 31°C.

The inbred lines OR and SAC generally appear to be very variable in response to different temperatures. The mass inbred BIK line is much more stable as shown by the average results for all temperatures. Furthermore, combining all

TABLE 2

Mean number of adults reared from 150 larvae at various temperatures from three inbred lines, their F_1's and F_2's with appropriate standard errors (see text)

Inbreds	15°C	24°/15°C*	24°C	24°/31°C	31°C	15°/31°C	Mean
OR	65 ± 15	90 ± 26	96 ± 19	58 ± 18	8 ± 4	69 ± 15	65 ± 33
SAC	90 ± 17	92 ± 14	86 ± 24	49 ± 14	17 ± 8	41 ± 11	63 ± 33
BIK	124 ± 18	127 ± 16	135 ± 17	106 ± 20	111 ± 21	120 ± 11	120 ± 20
F_1's							
OR × SAC	80 ± 16	80 ± 9	107 ± 8	88 ± 10	87 ± 26	76 ± 19	86 ± 19
SAC × OR	94 ± 20	73 ± 18	98 ± 12	72 ± 15	52 ± 11	62 ± 10	75 ± 22
BIK × OR	134 ± 15	133 ± 13	130 ± 14	128 ± 17	123 ± 23	98 ± 20	124 ± 21
OR × BIK	132 ± 16	126 ± 12	135 ± 14	125 ± 10	130 ± 12	104 ± 11	125 ± 16
BIK × SAC	119 ± 15	111 ± 19	132 ± 14	109 ± 14	111 ± 20	96 ± 23	113 ± 21
SAC × BIK	72 ± 17	58 ± 16	75 ± 17	47 ± 8	38 ± 9	48 ± 9	56 ± 19
F_2's							
OR × SAC	113 ± 22	127 ± 10	133 ± 16	107 ± 22	84 ± 13	101 ± 13	111 ± 23
SAC × OR	119 ± 20	125 ± 16	135 ± 11	111 ± 10	101 ± 21	101 ± 13	115 ± 20
BIK × OR	120 ± 19	109 ± 14	142 ± 15	137 ± 17	121 ± 13	113 ± 18	124 ± 20
OR × BIK	130 ± 13	122 ± 19	132 ± 17	119 ± 19	124 ± 8	124 ± 17	125 ± 16
BIK × SAC	117 ± 24	121 ± 17	127 ± 21	102 ± 19	122 ± 24	90 ± 14	113 ± 23
SAC × BIK	128 ± 16	130 ± 15	146 ± 10	91 ± 37	70 ± 25	109 ± 20	112 ± 33
Mean for each temperature	109 ± 28	108 ± 28	120 ± 26	96 ± 33	86 ± 43	90 ± 29	

* This represents a fluctuating temperature. For eight hours of a day, larvae were exposed to 24°C and for 16 hours to 15°C. For the other two fluctuating temperatures, 24°/31°C and 15°/31°C, the first temperature represents the eight hour period, and the last the 16 hour period.

temperatures, almost twice as many flies emerge in BIK as in OR and SAC. Once again this is probably due to the difference between mass inbreeding and sib-mating for the inbreeding system. The relative stability of the BIK inbred line as compared with OR and SAC is obvious from the relative magnitudes of the standard errors of the marginal means. Hence, it may be postulated that the BIK inbred line is more homeostatic than the OR and SAC inbred lines.

As might be expected, the two F_1 hybrids between OR and SAC are less variable in response to the temperature regimes than are the parental inbred lines. There is little evidence for a maternal effect, although the SAC × OR emergence is somewhat poorer than for OR × SAC. Using the mean of the two reciprocal F_1's, the possible existence of heterosis may be tested (Table 3). At the most unfavorable temperature, 31°C, there is very strong positive heterosis. Two other cases of positive heterosis occur (24°/31°C and 24°C). At 24°/15°C there is a degree of negative heterosis. Generally, there is a greater difference between the F_1 mean and the mid parental mean at the more unfavorable temperatures. The between temperature variability of the F_1 is much smaller than that for the two inbred lines.

Turning now to the F_1's between OR and BIK, it is obvious that there is no maternal effect (Table 2). Slight heterosis is present in some crosses and BIK is

dominant in the others. The variance of the marginal means for these crosses corresponds to that for the F_1's between OR and SAC (Table 2).

There was a large maternal effect between the hybrids BIK × SAC and SAC × BIK. The emergence of the hybrid SAC × BIK was much lower than BIK × SAC. For BIK × SAC there was, on the whole, no heterosis, and for SAC × BIK negative heterosis. As expected, the variances of these F_1's are low.

The F_2 crosses all produced similar numbers of adult flies. The maternal effect observed between BIK and SAC in the F_1 was eliminated, and the F_2's between OR and SAC gave more offspring than the F_1 hybrids. The variance components are, if anything, a little greater than the variances of the F_1's indicating the effect of F_2 segregation.

In Table 4, the analysis of variance of the data presented in Table 2 is given.

TABLE 3

Heterosis in the F_1 between OR and SAC

Temperature	Mean of reciprocal F_1's	Comment
15°C	87	SAC partially dominant
24°/15°C	76.5	negative heterosis
24°C	102.5	positive heterosis
24°/31°C	80	positive heterosis
31°C	69.5	positive heterosis
15°/31°C	69	OR completely dominant

TABLE 4

Analysis of variance of results in Table 2

	M.S.	F*	d.f.
Inbreds			
Genotypes	65,156	226.0	2
Temperatures	15,800	54.8	5
Genotypes × temperatures	2,492	8.6	10
Error	288		162
F_1's			
Genotypes	48,614	202.3	5
Temperatures	7,782	32.4	5
Genotypes × temperatures	697	2.9	25
Error	240		324
F_2's			
Genotypes	2,350	7.2	5
Temperatures	8,662	26.4	5
Genotypes × temperatures	1,619	4.9	25
Error	329		324
Interaction between inbreds, F_1's and F_2's	78,343		2
Total			899

* All the F values are significant at $P<0.001$.

The analysis is in three sections, inbred lines, F_1's and F_2's. Within each section there is a large temperature effect. A large genotype effect is present in the inbred lines and F_1's, but is smaller in the F_2 generation, as is obvious from the approximate equality of the numbers in the F_2 crosses (Table 2).

The relative magnitude of the mean squares for the three genotype × temperature interactions is of interest. The interaction is greatest for the inbred lines and smallest for the F_1's, the difference being significant ($P<0.05$). This shows that, as predicted from Table 2, the F_1's are more stable in response to the different environments than the inbreds. Thus, once again, it is shown that the F_1's show a higher degree of homeostasis than the inbreds. The F_2's have a genotype × temperature interaction intermediate in magnitude between the F_1's and inbred lines, as might intuitively be expected because of segregation in the F_2 generation.

Finally, there are two degrees of freedom which test the interaction between the inbred lines, F_1, and F_2 generations which is, as expected, highly significant when compared with the three error mean squares by means of the F test. This merely shows that the mean emergence of the inbred lines, F_1's and F_2's differs.

DISCUSSION

Basically, in this study, the adaptation of various inbred lines, their F_1 hybrids and F_2's, to a multiplicity of artificial environments was being investigated. Generally it was found that the heterozygous F_1 generation is less variable than the inbred parents. The stability of the F_1 generation was probably due to the superior homeostatic ability conferred upon it as a result of its heterozygosity. Evidence of this type has been presented by many authors among whom may be cited CLARINGBOLD and BIGGERS (1955), DOBZHANSKY and WALLACE (1953). LERNER (1954), ROBERTSON and REEVE (1955) and YOON (1955).

LERNER (1954), LEWONTIN (1956) and THODAY (1953) and others have stressed that characters of adaptive significance should be used in measuring homeostasis. Stability of fitness over all environments is the criterion of homeostasis. It is often difficult, for example, to see the relationship between some morphological characters and adaptive significance. LEWONTIN (1956) demonstrated that there is no simple relation between morphological uniformity and homeostasis for abdominal bristle number. Hence a population which exhibits a high degree of homeostasis is one in which characters of adaptive significance are kept constant under variable environmental conditions. However, the variability of other characters not directly related to fitness may be increased, as has been demonstrated for eye pigmentation in *Ephestia kühniella* by CASPARI and GOTTLIEB (1959). The ability of larvae to emerge as adults is obviously related to fitness and hence inferences on the relative variability of heterozygotes and homozygotes as representing different degrees of homeostasis ought to be tolerably valid.

The superior homeostatic ability of the F_1 hybrids is shown to lead, as expected, to a relatively small genotype × environment interaction when compared with the inbred lines. The F_2 generation occupies a position between the F_1's and in-

bred lines. A few environments will therefore give an estimate of the over-all behavior of the F_1, but many more environments will be needed for inbred lines. The widespread use of inbred lines suffers from the defect of considerable variation between environments. CLARINGBOLD and BIGGERS (1955) and MCLAREN and MICHIE (1956) have pointed this out for inbred lines of mice.

Many genotype-environmental interactions have been reported in the literature, and the problem has been discussed theoretically (COMSTOCK 1955; DEMPSTER 1955; LERNER 1958; and MATHER 1955). It is of great interest to both the evolutionist and the breeder to estimate the magnitude of the genotype-environmental interactions resulting from subtle, but nevertheless important micro-environmental changes. PARSONS and ALLARD (in press) present data on seed size in the lima bean (*Phaseolus lunatus* L.) grown in an apparently constant environment over a period of six to eight years demonstrating significant interactions between genotype and environment. All these studies indicate the need to study populations in many environments if predictions of possible progress under selection are to be reliable. The data reported in this paper emphasize the differential behavior of inbred and noninbred individuals in different environments and show that predictions made on heterozygous individuals exhibiting homeostasis would be more reliable than nonhomeostatic inbreds.

Finally, these data bring out an interesting contrast between mass inbreeding and inbreeding by sib-mating. The mass inbred BIK strain is shown to be almost equivalent in homeostatic ability to the F_1 hybrids. This shows up at extreme temperatures such as 31°C when BIK emergence is much higher than the OR and SAC emergence. Thus the mass inbred BIK line is better buffered against environmental extremes than the OR and SAC inbred lines. It is unlikely that this is due to the environments to which these inbred lines were subjected before bringing them into the laboratory, as all three inbred lines have been under constant temperature conditions for approximately ten years. The more likely interpretation is that mass inbreeding permits the unconscious selection of heterozygotes more readily than under sib mating. The hatchability figures confirm this interpretation, for these were all obtained at 24°C, so that the original environment of the BIK strain can have little or no effect.

In chickens, SCHULTZ (1953) found that the inbred lines were more sensitive, and the crosses between them less sensitive to environmental changes than the more or less randomly bred birds of production flocks. Similarly MCLAREN and MICHIE (1956) found that randomly bred Mousery mice were almost as uniform as the F_1 hybrids and the inbred lines more variable than the Mousery mice. In the randomly bred chickens and Mousery mice there is probably an unconscious selection of fitter heterozygotes as has been suggested for the mass inbred BIK line.

SUMMARY

1. Three inbred lines of *Drosophila melanogaster* were crossed in all combinations and the F_2 generation grown. Hatchability estimates were made, and the

ability of 150 larvae to emerge as adults under six different temperature regimes studied.

2. The hatchability of the eggs from the F_1 flies exhibited heterosis and decreased variability compared with the inbred lines. Thus homeostasis, superior to the inbred lines, is indicated.

3. The inbred lines were more variable than the F_1 hybrids in response to temperature treatment as measured by the emergence of 150 larvae as adults. Furthermore, the inbred lines exhibited a larger genotype-environmental interaction than the hybrids. The hybrids therefore have a better homeostatic mechanism than the inbred lines.

4. Two of the inbred lines (OR and SAC) were inbred by sib mating, and the third (BIK), by mass inbreeding. The BIK inbred line was almost equivalent to the hybrids in homeostatic ability, probably because of the greater opportunity for the unconscious selection of favorable heterozygotes.

5. Some of the implications of the results are discussed.

ACKNOWLEDGMENTS

I would like to thank Dr. E. R. Dempster of the University of California, Berkeley, who supplied the Oregon and Sacramento inbred lines, Dr. J. M. Carpenter of the University of Kentucky who supplied the Bikini inbred line, Dr. M. M. Green of the University of California, Davis, who made facilities available to carry out the experiments, and Dr. R. W. Allard of the University of California, Davis, for critically reading the manuscript.

LITERATURE CITED

Caspari, E., and F. J. Gottlieb, 1959 On a modifier of the gene *a* in *Ephestia kühniella*. Z. Ind. Abst. Vererb. **90**: 263–272.

Claringbold, P. J., and J. D. Biggers, 1955 The response of inbred lines to oestrogens. J. Endocrinol. **12**: 9–14.

Clough, M., and A. G. Cock, 1957 Variability of inbred and incross chickens. Nature **179**: 1030–1031.

Comstock, R. E., 1955 Theory of quantitative genetics: synthesis. Cold Spring Harbor Symposia Quant. Biol. **20**: 93–102.

Dempster, E. R., 1955 Maintenance of genetic heterogeneity. Cold Spring Harbor Symposia Quant. Biol. **20**: 25–32.

Dobzhansky, Th., and B. Wallace, 1953 The genetics of homeostasis in Drosophila. Proc. Natl. Acad. Sci. U. S. **39**: 162–171.

Lerner, I. M., 1954 *Genetic Homeostasis*. Oliver and Boyd. Edinburgh.
1958 *The Genetic Basis of Selection*. John Wiley and Sons. New York.

Lewontin, R. C., 1956 Studies on homeostasis and heterozygosity. Am. Naturalist **90**: 237–255.

Mather, K., 1955 Response to selection. Cold Spring Harbor Symposia Quant. Biol. **20**: 158–165.

McLaren, A., and D. Michie, 1956 Variability of response in experimental animals. A comparison of the reactions of inbred, F_1 hybrid and random bred mice to a narcotic drug. J. Genet. **54:** 440–455.

Parsons, P. A., and R. W. Allard Seasonal variation in lima bean seed size: An example of genotypic-environmental interaction. Heredity (in press).

Robertson, F. W., and E. C. R. Reeve, 1955 Studies in quantitative inheritance. Z. Ind. Abst. Vererb. **86:** 424–458.

Schultz, F. T., 1953 Analysis of egg shape of chickens. Biometrics **9:** 336–353.

Thoday, J. M., 1953 Components of fitness. Symposia Soc. Exptl. Biol. **7:** 96–113.

Yoon, C. H., 1955 Homeostasis associated with heterozygosity in the genetics of time of vaginal opening in the house mouse. Genetics **40:** 297–309.

CYTOGENETIC STUDIES OF PRECOCIOUS MEIOTIC CENTROMERE DIVISION IN LYCOPERSICON ESCULENTUM MILL.[1]

CARL D. CLAYBERG

Department of Genetics, Connecticut Agricultural Experiment Station, New Haven, Connecticut

Received June 15, 1959

IN the normal course of meiosis chromosome division is completed at the beginning of AII and the sister chromatids migrate to opposite poles. When division occurs earlier, it is regularly associated with the presence of unpaired chromosomes in late PI or at MI and is very likely stimulated by this lack of pairing. Rarely precocious division may take place in the absence of reduced pairing.

Unpaired meiotic chromosomes are present in organisms deviating from the diploid chromosome complement. in species hybrids with reduced chromosome homology, and in asynaptics. At AI in monosomics the univalent divides in almost all cells (OLMO 1936; SEARS 1952) or in about half of them (MORRISON 1953). The univalents of haploids usually segregate randomly to the poles during AI dividing rarely at this stage (BELLING 1927; IVANOV 1938; LEVAN 1942, 1945). In a few haploids the unpaired chromosomes divide frequently in AI (GAINES and AASE 1926; LESLEY and FROST 1928). The reduction of pairing in trisomics, polyploids, and species hybrids can lead to univalents at MI, but their behavior as to time of division is often quite irregular even in the same material (LESLEY 1928; MCCLINTOCK 1929; UPCOTT 1935).

Some univalents may lag and divide at AI or TI in partial and complete asynaptics, but this division usually does not occur until AII. There are, however, a few striking examples of precocious chromosome division associated with asynapsis. SMITH (1936) observed division of all univalents at AI in complete asynaptics segregating from the hybrid *Triticum monococcum* × *T. aegilopoides*. With the absence of bivalents at MI in partially asynaptic *Crepis capillaris*, all univalents sometimes divided during AI (RICHARDSON 1935). Regular division of all univalents took place at AI in asynaptic *Oenothera decipiens* regardless of their position on or off the metaphase plate (CATCHESIDE 1939). In contrast to Crepis the presence or absence of bivalents, which behaved normally, failed to influence this division.

Asynapsis in *Alopecurus myosuroides* (JOHNSSON 1944) represents a more extreme situation. Precocious division occurred at AI not only in all univalents but also in the bivalents, which sometimes had not yet completed chiasma separation. As with the previous case in Oenothera, a MII plate did not form and daughter univalents segregated randomly to the poles without dividing again.

[1] Adapted from a portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California. This work was supported in part by a fellowship from the University of California.

JOHNSSON (1944) believed that the aberrant behavior represented a true precocity rather than a retarded first division. He based this opinion on his study of chromosomes in other *A. myosuroides* mutants possessing retarded first division and concluded that in his asynaptic "the centromere division itself [distinct from the asynapsis] would seem to be subject to a genotypical variation." Here, as elsewhere used in the present account, "*centromere division*" refers to completion of chromosome division, for true centromere division may normally occur as early as AI (LIMA-DE-FARIA 1956).

In autotetraploid *Chrysanthemum atratum*, DOWRICK (1953) observed a high proportion of cells at MI having only bivalents. All the centromeres divided precociously during this stage in these cells, although sister chromatids did not fall apart until homologues had migrated to opposite poles at AI. Each daughter nucleus thus contained the unreduced chromosome number. The second division was completely suppressed in such cells. Normal microsporocytes in the same material had the chromosome associations and behavior typical of autotetraploids, the univalents dividing at AI. Reduced chromosome pairing and chiasma frequency and a delayed PI were associated with the centromere precocity.

LAMM (1944) has briefly reported a rare instance in which precocious chromosome division occurring in TI was apparently not caused either by asynapsis or by an unbalanced chromosome complement. A sterile mutant in *Lycopersicon esculentum* appeared in segregating progenies which suggested inheritance by a single recessive factor. Anthers examined from one of the sterile plants showed meiosis to be normal during diakinesis and MI, with homologous chromosomes segregating regularly to opposite poles at AI. Chromosomes at TI "arranged themselves at each pole into a plate, whereupon their centromeres divided." The resulting TI groups thus contained the somatic chromosome number. The daughter chromosomes became oriented on two plates at MII and segregated to the poles at random in AII without further division. Microspore quartets usually resulted despite irregular chromosome distribution. No fertile pollen was produced. Although the aberrant plant analyzed cytologically was also trisomic, later observations of diploid segregants indicated that the precocity was not due to the extra chromosome (LAMM, unpublished).

The present study was initiated to determine the nature of another sterile tomato mutant whose infertility appeared to be unassociated with asynapsis or chromosome unbalance (CLAYBERG 1958a). Cytological analysis of later generations revealed that the sterility was caused by precocious centromere division begun late in the first meiotic division, mostly completed by PII, and resulting in irregular chromosome segregation at AII.

MATERIAL

The mutant originated from a partially sterile plant found in 1953 by DR. CHARLES M. RICK of the University of California at Davis. This plant appeared in a commercial field of the tomato variety Earlypak.

Inheritance

A few seeds set by open pollination on the original plant gave rise to a family containing both fertile and pollen-sterile individuals. Some of the pollen-sterile plants were crossed with a highly inbred strain of Pearson, a variety quite similar in general habit to Earlypak. Pollen-sterile plants in the resulting progeny were backcrossed by Pearson. A probable simple trisomic (56L130–1) from this backcross and a fertile diploid (56L111–5) from the first cross with Pearson were progeny tested using open pollinated seed. Cytological studies initiated in these two test progenies revealed the presence of sterile diploids possessing the meiotic abnormality to be described.

Both of these test families were grown from seed of open pollinated fruit but may be regarded as selfed progenies. RICK (1948) has found that the rate of natural cross-pollination in male-sterile mutants at Davis is quite low, ranging from one to seven percent. In addition plant 56L130–1 was grown in an isolated male-sterile crossing plot where the only available pollen was the little the plant itself produced. The seed from this plant gave rise to family 57L501, containing 70 plants which were examined cytologically. Twenty-eight of these possessed the meiotic precocity and had 90–100 percent aborted pollen. Ten of the 28 were also simple trisomics. The 42 plants lacking the precocity included 13 trisomics. The extra chromosome in all cases probably was chromosome 4 on the basis of plant habit (RICK and BARTON 1954).

This family contributes little evidence for the inheritance of the precocity. The χ^2 value calculated for monofactorial recessive determination of the aberrancy was 8.40 with $p = 0.006$. It does not appear that the trisomy is affecting the inheritance of the precocity, for a contingency test suggests no association between the two ($\chi^2 = 2.56$, $p = 0.11$).

Family 57L506 from the fertile diploid 56L111–5 provides more satisfactory information. Cytological examination showed that 14 of the 50 plants grown in this family were diploids with the precocious centromere division and 90–100 percent pollen abortion. The remaining 36 were normal fertile diploids. This ratio satisfactorily fits inheritance by a single recessive gene ($\chi^2 = 0.24$, $p = 0.64$).

Part of this family was grown in the greenhouse and not maintained after cytological identification. Open pollinated seed collected at Davis from the 27 fertile plants in the field grown portion of the family was planted in the summer of 1958 at Mt. Carmel, Connecticut.

The complete correlation in families 57L501 and 57L506 between the meiotic precocity and greater than 90 percent pollen abortion has already been mentioned. This correspondence permitted identification of aberrant plants in the 1958 plantings by acetocarmine pollen smears, using a minimum of three flowers per plant. Nine of the 27 families were nonsegregating, containing 18–20 fertile plants each. The observed proportion of segregating to nonsegregating families thus gives an exact fit to the 2:1 ratio expected. Table 1 shows the results for the 18 segregating families. The summation χ^2 value of 24.24 ($p = 0.15$) is com-

patible with the assumption that all of these families are segregating in a 3:1 ratio.

On the basis of this evidence the symbol *pc* is proposed for the single recessive gene controlling the centromere division precocity. No expression of this gene was observed other than its action on meiosis.

Cytology

Cytological analysis was confined to microsporocytes which were fixed in 1:3 acetic alcohol, mordanted in iron alum, and stained with acetocarmine as described by Soost (1951). The technique was modified by briefly heating the stained material over a steam bath before smearing (Barton 1950).

All counts and photographs are from temporary slides ringed with approximately equal parts of paraffin and anhydrous lanolin. These, being miscible, may be kept melted in a test tube in the steam bath and applied to the margins of the cover slip with a small brush.

The course of meiosis in normal material has already been characterized by Humphrey (1934). Both the fertile and aberrant diploids used for the following photographs come from families 57L501 and 57L506.

Prophase and metaphase I: Throughout these stages chromosome behavior appears to correspond in all respects to that of fertile plants. Initiation of pairing during zygotene seems in no way affected, and pachytene pairing is complete

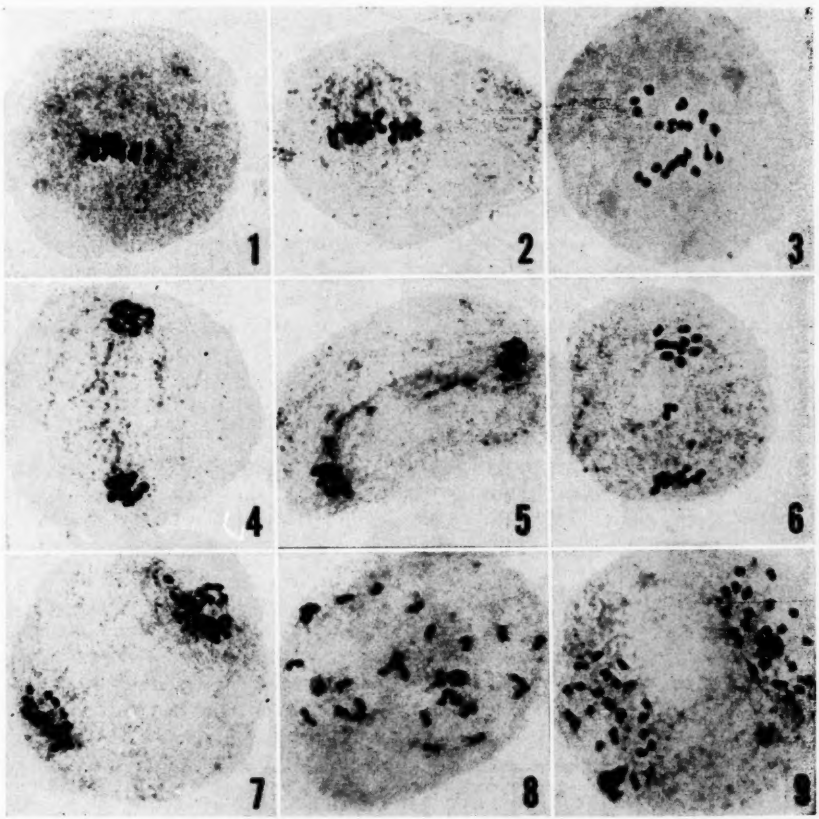
TABLE 1

Frequency of plants with precocious centromere (pc) *division in segregating F_2 progenies from family 57L506*

Family	*pc*	Total	χ^2(3:1)
58L52	3	20	1.07
58L54	6	18	0.67
58L55	2	18	1.85
58L56	8	20	2.40
58L57	3	19	0.85
58L58	3	20	1.07
58L63	1	20	4.27
58L64	4	20	0.27
58L65	1	20	4.27
58L67	3	20	1.07
58L69	5	20	0.00
58L70	6	19	0.44
58L71	3	20	1.07
58L73	5	16	0.33
58L74	5	19	0.01
58L75	5	18	0.08
58L76	8	20	2.40
58L77	2	19	2.12
			$\chi^2_s = 24.24$
	d.f. = 18	p = 0.15	

with normal bivalent configurations maintained through MI (Figures 1 and 2). It is quite possible, however, that chiasma frequency could be reduced somewhat without altering bivalent associations. To verify this chiasma counts might be taken at diakinesis, since diplotene in tomato is unfavorable for observation (Soost 1951).

The presence of both trisomic and diploid *pc* plants in family 57L501 makes possible an alternative and simpler technique. If *pc* reduces chiasma frequency



FIGURES 1–9.—First metaphase to second prophase 740×. FIGURE 1.—First metaphase from a normal plant. FIGURE 2.—First metaphase from mutant *pc*. FIGURE 3.—First anaphase in a normal plant. FIGURE 4.—First telophase in a normal plant. FIGURE 5.—First telophase from *pc* with a lagging and dividing daughter bivalent and another lagging chromosome. FIGURE 6.—First telophase in *pc* with lagging chromosomes and showing undivided chromosomes in the upper nucleus. FIGURE 7.—First telophase in *pc* showing some division of daughter bivalents in the two nuclei. FIGURE 8.—Second prophase from a normal plant. FIGURE 9.—Second prophase in *pc* having 24 chromosomes in each daughter nucleus.

slightly, there should be a higher proportion of bivalent-univalent associations, instead of trivalents, in a trisomic homozygous for this gene than in an otherwise normal trisomic.

The results in Table 2 suggest that *pc* might have a slight effect in this direction, but the contingency χ^2 value for the data is 0.34 ($p = 0.58$). This indicates that no significant differences exist between trivalent frequency in *pc* and non-*pc* plants and consequently that chiasma frequencies are quite likely the same in the two types.

Anaphase and telophase I: The chromosomes of the mutant first deviate from normal behavior at late anaphase. Although bivalents separate regularly, some of the resulting half-bivalents frequently lag and may divide (Figures 5 and 6). Figure 10 shows a cell at PII in which such a chromosome appears to have lagged and divided at AI with one of the sister chromatids being included in a telophase nucleus and the other remaining in the cytoplasm. Further observations on the frequency of lagging and division of half-bivalents at AI will be included in the description of PII.

It is not entirely clear when the centromeres begin their precocious division, aside from those that have split subsequent to chromosome lagging. The chromosomes in the upper group of the cell in Figure 6 have not divided by early telophase. But one or more chromosomes at the poles are divided in the telophase cell of Figure 7. These chromosomes may have lagged, divided, and finally reached the poles; or they may have completed migration before dividing. In any case the centromeres of most chromosomes have divided by PII in all cells observed. Much of this division very likely occurs during interkinesis.

The chromosomes do not form a plate at TI and then divide, as reported by LAMM (1944) for a similar mutant in tomato.

Prophase II: Cells in this stage (Figures 9 and 10) differ markedly from those of normal material (Figure 8), for most of the centromeres are clearly divided. Even when a few sister chromatids are conjoined, their points of attachment seem more attenuated than in chromosomes at the same stage in fertile plants (cf. Figures 8 and 10). The separated chromosomes have some tendency to be grouped two-by-two. This may be due to failure of movement following division, or forces of attraction may be acting to keep the chromosomes so oriented. The associations probably involve homologues, but verification of this has not been possible.

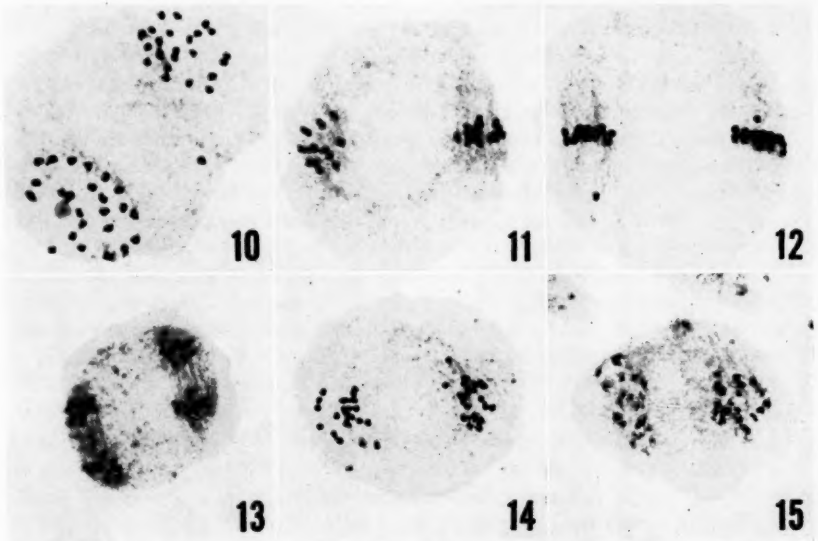
Table 3 records the frequency of various chromosome distributions at PII. No record was made of occasional incompletely separated sister chromatids, because

TABLE 2

Chromosome associations in trisomics with and without precocious centromere division

	Number of cells counted			
	III	II–I	3I	Total
57L501–7 (*pc*)	56	44	0	100
57L501–9 (*pc*$^+$)	60	40	0	100

doubt existed as to whether they represented failure of division or chance contact in smearing. The proportion of cells with chromosomes excluded from the second prophase nuclei is 53 percent. A more extensive count of 411 cells in PII and MII on the same slide indicated that 41.3 percent contained micronuclei.



FIGURES 10–15.—Second prophase to second telophase 740×. FIGURE 10.—Second prophase in *pc* showing 24 chromosomes in one nucleus, 23 in the other, and one chromosome in the cytoplasm. Some of the chromosomes are not completely separated. FIGURE 11.—Second metaphase from a normal plant. FIGURE 12.—Second metaphase in *pc* with two chromosomes off the plates but in the spindles. FIGURE 13.—Second anaphase from a normal plant. FIGURE 14.—Second anaphase in *pc* showing much chromosome lagging. FIGURE 15.—Double restitution nucleus formation in *pc* plus a micronucleus.

TABLE 3

Chromosome distributions at second prophase in microsporocytes of a diploid pc *plant (57L501–68)*

Number of daughter chromosomes excluded from prophase nuclei													
0		1		2		3		4		7			
Nuc. counts*	F.	Nuc. counts	F.	Nuc. counts	F.	Nuc. counts	F.	Nuc. counts	F.	Nuc. counts	F.		
24–24	10	23–24	6	23–23	2	22–23	1	21–23	1	20–21	2		
23–25	1	22–25	1	22–24	1			20–24	1				
22–26	2	20–27	1										
20–28	1												
Total	14		8		3		1		2		2		

Total number of cells = 30

Cells having excluded chromosomes = 53 percent

* Nuc. counts = Nuclear counts.
F = frequency.

Those cells in Table 3 having one or both nuclei with odd numbers of daughter chromosomes may be used to estimate the minimum frequency of precocious chromosome division in AI. The data indicate that this event occurs in at least one chromosome for every two cells.

Metaphase II: All chromosomes are *regularly* oriented on the metaphase plates (Figure 12). Frequently one to several chromosomes are in the spindles but off the plates. These chromosomes may have failed to migrate to the plates, or they may have precociously initiated poleward movement. Chromosomes excluded from the TI daughter nuclei appear to remain out of the MII spindles.

At this stage the chromosomes are associated two-by-two, as they are during PII. This continued association therefore seems to eliminate failure of chromosome movement as an explanation for the association in PII. Although in lateral view (Figure 12) plates superficially resemble those of normal material, the chromosomes are clearly divided when the plates are viewed in polar position.

Anaphase and telophase II: Chromosome segregation to the poles is random without further division (Figure 14). Many of the chromosomes tend to lag in the center of the spindle. As a consequence restitution nuclei frequently form from one or both spindle groups (Figure 15). Table 4 gives "quartet" counts for two diploid *pc* plants.

Unquestionably a significant difference exists between them: 57L501–68 has a mean of 2.36 spores per group while the mean for 57L501–69 is 3.67.

Family 57L501, as mentioned earlier, resulted from two consecutive crosses of Earlypak by Pearson. Although these varieties are similar in general plant habit, they differ sufficiently for a number of genes to be segregating in backcross progenies. Some of these genes might reasonably be expected to modify the frequency with which second division restitution nuclei occur in different plants homozygous for *pc*.

Fertility: The proportion of stainable pollen in all examined *pc* plants varied from zero to ten percent. Some stainable pollen is probably functional, since tetraploids appeared in the progeny of the original partially sterile accession. Furthermore, cytological examination of one of these tetraploids proved it to be *pc*.

The difference in restitution nucleus formation between plants 57L501–68 and 57L501–69 does not seem to affect appreciably the proportion of stainable pollen grains they produce. Examinations were made of a total of six flowers for each plant collected over a period of four months, and a minimum of 500 grains per flower were counted. The amount of stainable pollen for 57L501–68 ranged from 0.00 to 4.94 percent and for 57L501–69 from 0.19 to 3.23 percent.

TABLE 4

Distribution of "quartet" types in diploid plants with precocious centromere division

	Number of spores per "quartet"						Total "quartets"
	2	3	4	5	6	7	
57L501–68	234	89	11	3			337
57L501–69	39	136	167	56	8	2	408

Since diploid grains should be functional, one possible explanation of the high pollen sterility in 57L501–68 might be unfavorable action of *pc* on the mitotic division of the microspore. The strongly granular nature of the cytoplasm at this stage in tomato discourages investigation of this possibility. In view of the high proportion of chromosomes excluded from the daughter nuclei, as indicated in Table 3, it seems more probable that the abortion of these "diploid" spores is due to degeneration resulting from chromosome unbalance.

Ovule fertility is also reduced. Twenty-one backcross pollinations of 57L501–68 and 57L501–69 by normal Earlypak all yielded fruit, 17 being parthenocarpic. The remaining four fruits contained a total of 14 seeds, only one of which germinated. The resulting plant, 57L521–1, was triploid and non-*pc*. Presumably it developed from the union of an unreduced egg from the *pc* parent, 57L501–68, and a normal haploid sperm of Earlypak.

Megasporogenesis was not studied cytologically in *pc* plants. But their apparent formation of diploid megaspores, as indicated by the triploid 57L521–1 and the tetraploids previously mentioned, plus their high ovule infertility imply that meiosis proceeds similarly in both megasporocytes and microsporocytes. Simple and multiple trisomics identified in the Pearson backcross progenies demonstrated that *pc* mutants can also produce functional hyperploid megaspores. The chromosome unbalance probably is a consequence of irregular segregation at either the first or the second meiotic division.

DISCUSSION

The observed meiotic aberrancies of *pc* mutants may all be attributed to a division precocity of the chromosome regions normally holding sister chromatids together until AII. LIMA-DE-FARIA's (1956) studies suggest that the regions involved are the most proximal portions of the chromosome arms rather than the centromere itself. In *Agapanthus umbellatus*, *Fritillaria meleagris*, *Rhoeo discolor*, and *Mecostethus grossus* he found that the centromeres are divided and well separated from each other at pro-metaphase II. He observed such division as early as AI in *Tradescantia virginiana*, *T. bracteata*, and *Secale cereale*. The small size of tomato chromosomes at stages subsequent to PI makes verification of his results difficult in this material.

In some mutants centromere precocity exhibited during the first meiotic division does not seem to continue into the second division. Daughter univalents thus fail to congress to the MII plates, as in asynaptic *Oenothera decipiens* (CATCHESIDE 1939). If the precocity does continue, the daughter univalents can go to the plates but not divide. This happens in asynaptics of *Lycopersicon esculentum* (SOOST 1951) and is typical of the *pc* mutant.

Daughter univalents also can divide following metaphase plate congression. This is unlikely to occur, however, since it involves two equational divisions in meiosis instead of the customary one. EKSTRAND (1932) has reported that such behavior is customary in daughter univalents of partially asynaptic barley, although the division follows AII separation of the unaffected chromosomes. One

other instance of regular double division of univalents at meiosis has been found (FEDERLEY 1931).

If the precocity of *pc* is expressed before AI. it appears to have no observable effect on chromosome pairing and chiasma formation. During AI the precocity occurs initially as a bipolarization of some chromosomes, following separation of homologues. These chromosomes lag and frequently divide in the first meiotic division. Otherwise most of the precocious division seems to take place during interkinesis. This conclusion follows from the observation that centromeres are often undivided at TI, while by PII the great majority of them are split.

Although the aberrant centromere behavior in *pc* has been interpreted as precocity, the possibility also exists that the centromere division time cycle is normal while that of the chromosomes is delayed. Precocity is the preferred explanation because there are no obvious differences in corolla length of buds from *pc* and normal plants containing microsporocytes in the same stages of meiosis. Studies of normal material in connection with an asynaptic mutant (CLAYBERG 1958b) having delayed meiosis showed that such measurements provide a fairly reliable means of ascertaining the meiotic stages in the respective buds.

The two-by-two chromosome associations seen at PII and MII are difficult to understand. One might be tempted to compare them with bivalents at MI in normal material, except for the presence of chiasmata in the latter. The close synapsis of homologues in bivalents where no chiasmata are observed (HUGHES-SCHRADER 1943) suggests that the forces holding bivalents together may be stronger than those acting on the second division *pc* chromosome associations.

The similarity between meiosis of *pc* plants and that of the tomato mutant reported by LAMM (1944) and described earlier in this paper is remarkable. Quite possibly the same locus may be involved in both. This unfortunately cannot be verified experimentally, since LAMM's cultures are no longer available.

The most marked resemblances between the two mutants are the precocious centromere division and orientation of the precociously divided chromosomes on the MII plates. LAMM's mutant differs from the present case in that his showed no lagging and dividing chromosomes at AI, formed plates at TI after which the centromeres divided, and usually gave rise to four spore "quartets."

The two varieties in which the aberrant types appeared seem to be completely unrelated, indicating that they represent independent mutations. Earlypak is a large-fruited, determinant tomato introduced about 1950 by the Ferry-Morse Seed Company. LAMM's mutant occurred in Danish Export, a long established variety with small fruits and indeterminate habit, supposedly developed in Denmark during the nineteenth century.

SUMMARY

The almost complete sterility of a mutant in the tomato variety Earlypak results from an abnormality of meiosis controlled by a single recessive gene *pc*. The aberrant behavior is characterized by the regular occurrence of precocious meiotic centromere division. The precocity first appears at anaphase I in some daughter

bivalents which often lag and divide. The centromeres of those chromosomes not lagging in the first division divide in most instances by prophase II. This division probably takes place during interkinesis. All chromosomes *regularly* become oriented on the metaphase II plates. The precociously divided chromosomes migrate to the poles at random without further division. The considerable amount of chromosome lagging in the second division frequently results in the formation of restitution nuclei. Some of the diploid spores so produced are functional and give rise to polyploid offspring. Irregular chromosome segregation also results in gametes of unbalanced chromosome number as reflected in the appearance of trisomic progeny.

ACKNOWLEDGMENTS

The writer wishes to acknowledge his gratitude to DR. CHARLES M. RICK for providing the material studied and for his valuable advice during the course of the investigation and the writing of the manuscript.

LITERATURE CITED

BARTON, D. W., 1950 Pachytene morphology of the tomato chromosome complement. Am. J. Botany **37:** 639–643.

BELLING, J., 1927 The attachments of chromosomes at the reduction division in flowering plants. J. Genet. **18:** 177–205.

CATCHESIDE, D. G., 1939 An asynaptic Oenothera. New Phytologist **38:** 323–334.

CLAYBERG, C. D., 1958a Genetically determined precocious centromere division in *Lycopersicon esculentum* Mill. Proc. 10th Intern. Congr. Genet. **2:** 53.

1958b Cytogenetic studies of two meiotic abnormalities in *Lycoperscion esculentum* Mill. Unpublished Ph.D. dissertation. Univ. of California. Berkeley.

DOWRICK, G. J., 1953 The chromosomes of Chrysanthemum. III: Meiosis in *C. atratum*. Heredity **7:** 219–226.

EKSTRAND, H., 1932 Ein Fall von erblicher Asyndese bei Hordeum. Svensk Botan. Tidskr. **26:** 292–302.

FEDERLEY, H., 1931 Chromosomenanalyse der reziproken Bastarde zwischen *Pygaera pigra* and *P. curtula* sowie ihrer Rückkreuzungsbastarde. Z. Zellforsch. **12:** 772–816.

GAINES, E. F., and H. C. AASE, 1926 A haploid wheat plant. Am. J. Botany **13:** 373–385.

HUGHES-SCHRADER, S., 1943 Meiosis without chiasmata in diploid and tetraploid spermatocytes of the mantid *Callimantis antillarum* Saussure. J. Morph. **73:** 111–141.

HUMPHREY, L. M., 1934 The meiotic divisions of haploid, diploid, and tetraploid tomatoes with special reference to the prophase. Cytologia **5:** 278–300.

IVANOV, M. A., 1938 Experimental production of haploids in *Nicotiana rustica* L. Genetica **20:** 295–397.

JOHNSSON, H., 1944 Meiotic aberrations and sterility in *Alopecurus myosuroides* Huds. Hereditas **30:** 469–566.

LAMM, R., 1944 A case of abnormal meiosis in *Lycopersicum esculentum*. Hereditas **30:** 253. (Abstr.)

LESLEY, J. W., 1928 A cytological and genetical study of progenies of triploid tomatoes. Genetics **13:** 1–43.

LESLEY, M. M., and H. B. FROST, 1928 Two extreme "small" Matthiola plants: a haploid with one and a diploid with two additional chromosome fragments. Am. Naturalist **62:** 22–33.

LEVAN, A., 1942 Studies on the meiotic mechanism of haploid rye. Hereditas **28:** 177–211.
1945 A haploid sugar beet after colchicine treatment. Hereditas **31:** 399–410.

LIMA-DE-FARIA, A., 1956 The role of the kinetochore in chromosome organization. Hereditas **42:** 85–160.

MCCLINTOCK, B., 1929 A cytological and genetical study of triploid maize. Genetics **14:** 180–222.

MORRISON, J. W., 1953 Chromosome behavior in wheat monosomics. Heredity **7:** 203–226.

OLMO, H. P., 1936 Cytological studies of monosomic and derivative types of *Nicotiana tabacum*. Cytologia **7:** 143–159.

RICHARDSON, M. M., 1935 Meiosis in Crepis II. Failure of pairing in *Crepis capillaris* (L.) Wallr. J. Genet. **31:** 119–143.

RICK, C. M., 1948 Genetics and development of nine male-sterile tomato mutants. Hilgardia **18:** 599–633.

RICK, C. M., and D. W. BARTON, 1954 Cytological and genetical identification of the primary trisomics of the tomato. Genetics **39:** 640–666.

SEARS, E. R., 1952 Misdivision of univalents in common wheat. Chromosoma **4:** 535–550.

SMITH, L., 1936 Cytogenetic studies in *Triticum monococcum* L. and *T. aegilopoides* Bal. Univ. Missouri Agr. Exptl. Sta. Res. Bull. **248:** 1–38.

SOOST, R. K., 1951 Comparative cytology and genetics of asynaptic mutants in *Lycopersicon esculentum* Mill. Genetics **36:** 410–434.

UPCOTT, M. B., 1935 The cytology of triploid and tetraploid *Lycopersicum esculentum*. J. Genet. **31:** 1–19.

THE GENETICS OF PAPILIO DARDANUS, BROWN. I. RACE CENEA FROM SOUTH AFRICA

C. A. CLARKE AND P. M. SHEPPARD

Departments of Medicine and Zoology, University of Liverpool, Liverpool, England

Received June 17, 1959

THE evolution of mimicry, although it may depend on the occurrence of extensive genetic changes some of which took place a long time ago, may nevertheless often be analyzable in considerable detail. However, despite the fact that much work has been done on the theoretical side (FISHER 1930) little progress has been made by means of direct experiments. An investigation utilizing the techniques of genetics, ecology and ethology would not only solve many of the problems of the evolution of mimicry itself but might throw considerable light on the factors governing evolution in general.

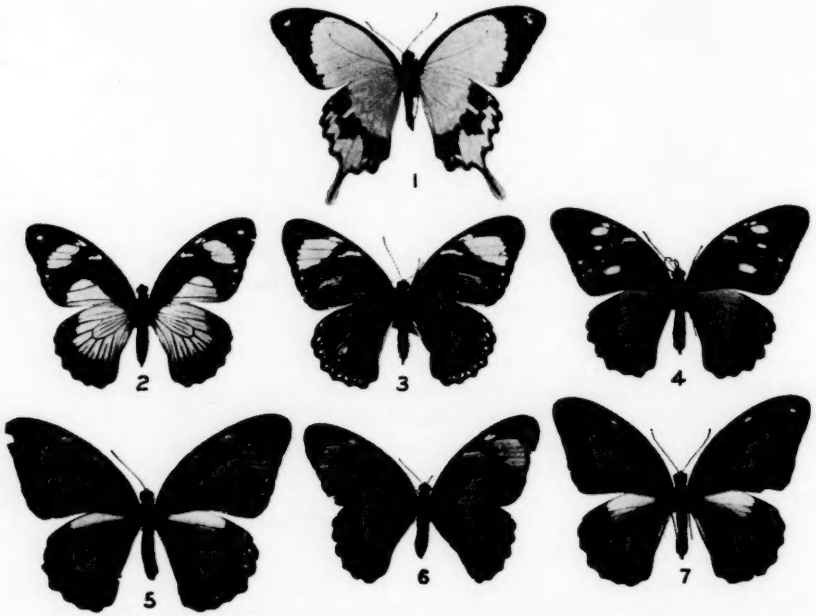
To investigate the genetic changes which have occurred during the evolution of mimicry it is necessary to make interrace and even interspecific crosses (FORD 1953). In order to obtain the maximum amount of information it is desirable to choose a species which has many mimetic and nonmimetic forms and in which some or all the mimetic forms are missing in some races. It is also necessary to know the geographical distribution of the races in detail together with the relative abundance of mimics and models in the various areas. The African swallowtail *Papilio dardanus* Brown, with its striking polymorphism in the female, fulfils these requisites better than most butterflies, and has been used in the present investigation.

This paper is the first of three dealing with the formal genetics of the various races of the insect. Only relevant broods are given in the tables but copies of the complete data, comprising over three thousand matings, are available on request.

Papilio dardanus, *race* cenea: This race inhabits South Africa, northwards to Delagoa Bay. The males are monomorphic, yellow, tailed and nonmimetic as they are wherever the species is found (Figure 1). The female forms that have been studied by us are the nonmimetic f. *leighi*, f. *natalica* and f. *salaami* and the mimics f. *hippocoonides*, f. *cenea*, f. *trophonius* (Figures 2–7) and a modification of f. *trophonius* in which the large apical spot on the forewings is buff and not the normal white (for a description of the forms, their models and their distribution see FORD 1936 and CLARKE and SHEPPARD 1959a). It should be noted that the name *cenea* is used to designate both the South African race and also one of the common mimetic female forms occurring within this and other races.

MATERIALS AND METHODS

Several well-known South African naturalists, MESSRS. GOWAN C. CLARK, C. G. C. DICKSON, D. A. SWANEPOEL and R. W. WELLS, have kindly sent us eggs, larvae and pupae of *P. dardanus cenea* from Natal.



FIGURES 1–7.—Forms of *P. dardanus* race *cenea*. FIGURE 1.—Male (light areas are pale yellow). FIGURE 2.—Female f. *hippocoonides* (light areas are white). FIGURE 3.—Female f. *trophonius* (light subapical spot is white, medium dark areas are bright orange). FIGURE 4.—Female f. *cenea* (light areas on forewing are white, base of hind wings buff). FIGURE 5.—Female f. *natalica* (light areas are all buff). FIGURE 6.—Female f. *salaami* (subapical spot light orange, medium dark areas are bright orange). FIGURE 7.—Female f. *leighi* (spots on forewing light orange, base of hind wing pale buff).

The method of breeding the butterflies in England is described by us in detail elsewhere (CLARKE and SHEPPARD 1959a,b) and since the earlier paper there have been two main alterations in the technique. Firstly, we have found that the caterpillars thrive much better on *Choisya ternata* (the Mexican orange flower) than on Citrus, and secondly, that the best way to have the material sent from Africa is in the form of living butterflies folded flat in cellophane envelopes.

RESULTS

Some observations on f. hippocoonides

This form which mimics the Danaid *Amauris niavius dominicanus* is very similar in pattern to f. *hippocoon* which occurs in other races of *dardanus* and which mimics *A. niavius niavius*, a different subspecies of the model for *hippocoonides*. In crosses between *hippocoon* and *hippocoonides* (in which the genotype of the male is known in this respect) we have found that there is no clear-cut

segregation in the F_2 (or equivalent) and backcross matings. This indicates that the two forms result from the modification of a single major gene (CLARKE and SHEPPARD 1960). Consequently, in this paper we have not distinguished between *hippocoonides*, *hippocoon* or hybrids between the two and all have usually been referred to as *hippocoonides*. The three types of insect serve equally the important purpose of providing broods homozygous for the white female form.

Inheritance of the forms of race cenea

As previously ascertained, (CLARKE and SHEPPARD 1959a), *cenea* is dominant to *hippocoonides* as is *leighi*, and neither is sex-linked. Further evidence of these dominance relationships is given by broods 1457, 1462, 1690, 1707, 2156, and 2185, where the male parent was homozygous for *hippocoonides*, *hippocoon* or the hybrid (Table 1).

It was previously known that *leighi* was probably dominant to *cenea* (CLARKE and SHEPPARD 1959a) and brood 1415 helps to strengthen this view since a *cenea* female mated to a male both of whose parents could have been heterozygous for *leighi* gave rise to seven *leighi* females, whereas if *cenea* were dominant at least a 1:1 ratio would be expected, the departure from this ratio being significant ($P>0.02$). This is confirmed by brood 2235, where a *cenea* female mated to a male heterozygous *leighi/trophonius* gave only *leighi* and *trophonius* offspring. Further evidence is provided by broods 1273 and 1386. In brood 1273, a female *leighi* which had not had f. *trophonius* in its known ancestry produced a female *trophonius* in its offspring indicating that the wild male to which it was mated was carrying *trophonius*. This female *trophonius* was mated to a male which had

TABLE 1

Dominance relationships of f. hippocoonides, *f.* leighi *and f.* cenea

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Males	*cenea* females	*hippocoonides* females	*trophonius* females	*leighi* females
1273	*leighi**	wild	0	0	0	1	0
	trophonius	male not carrying					
1386	1273	*cenea*	4	4	0	7	0
	cenea†	male offspring of					
1415	wild	*leighi* mother	6	0	0	0	7
1457	*cenea*	*hh*‡	10	1	2	0	0
1462	*leighi*	*hh*	11	0	5	0	5
1690	*cenea*	*hh*	24	10	7	0	0
1707	*cenea*	*hh*	7	9	0	0	0
2156	*leighi*	*hh*	11	0	5	0	6
2185	*leighi*	*hh*	3	0	1	0	3
	cenea	male from *salaami*					
2235	wild	family§	19	0	0	8	16

* Not known to have *trophonius* in its ancestry.
† f. *leighi* is so rare that a wild *cenea* is highly unlikely to be carrying the gene responsible for this form: moreover there is much subsequent evidence that *leighi* is nearly always dominant to *cenea*.
‡ The designation of the genotype of the male known to be homozygous for *hippocoonides*, *hippocoon* or the hybrid.
§ See Table 4.

no *cenea* in its known ancestry (brood 1386). The offspring of brood 1386 segregated *cenea* and *trophonius*. If, as seems probable from other evidence, *leighi*, *trophonius* and *cenea* are allelomorphs (see below) then the female *trophonius* 1273 must have been *trophonius/cenea* and therefore as she could not have inherited *cenea* from her father, for she received *trophonius* from him, she must have inherited it from her *leighi* mother, thus proving the dominance of *leighi* over *cenea* (see Table 1).

That the dominance relationship between *leighi* and *cenea* can occasionally be upset is shown by a consideration of broods 1372, 1466, 1634 and 1740, all of which are very closely related to one another. Brood 1372 had one *leighi* and three *cenea* females; one of the latter was highly abnormal in that although it had the *cenea* patterning (except that the spots near the apex of the forewing were larger and more circular than normal) the coloring was orange like that of *leighi*. This particular butterfly was mated (brood 1466) to a male with *cenea* but no *leighi* in its known ancestry. The offspring were four *cenea* females and one *leighi* female, but again one of the "*cenea*" had the orange spots like its mother. This butterfly was mated (brood 1634) to a brother, and again two "*cenea*" with orange spots were produced. One of these, when mated (brood 1740) to a male homozygous for *hippocoonides* (*hippocoon*) (and therefore not carrying *cenea* or *leighi*) produced a very abnormal looking *leighi*, the butterfly in some respect looking like f. *natalica*. The fact that the *cenea* which were heterozygous for *leighi* were decidedly abnormal, coupled with the fact that at least one of the *leighi* produced was itself highly abnormal, indicates that in these broods we have a modifier or modifiers which have broken down the normal dominance relationship and in fact nearly reversed them. Many other *leighi/cenea* heterozygotes must have existed (broods 1020, 1157, 1176, 1192, 1365 and 2235) but no other orange-spotted *cenea* have appeared showing that the pattern of these insects was abnormal and probably due to the presence of a single gene modifier (see Table 2).

That *trophonius* is not recessive to all other forms was shown in our previous paper (CLARKE and SHEPPARD 1959a). Subsequently broods 1680, 1705, 1792 and 1807, in which the male parent was known not to be carrying *trophonius* have shown that it is not sex-linked and that it is dominant to *hippocoonides* (and *hippocoon*). Moreover brood 1824, (among others) confirms this finding, for here a good 3:1 ratio of *trophonius* to *hippocoonides* was obtained. Furthermore, *trophonius* is dominant to *cenea* as is shown by broods 1591 and 1705 where a *trophonius* mated to a male not carrying *cenea* produced both *trophonius* and *cenea* (see Table 3). Brood 1386 (see Table 1) is also confirmatory.

The relationship between *trophonius* and *leighi* is an interesting one. The pattern of *salaami* is the same as that of *trophonius* except that the white apical patch on the forewing is replaced by the orange color found in this area in *leighi* (Figure 7). Moreover, *salaami* is very rare in South Africa. It has however appeared in our broods but only when both *leighi* and *trophonius* are also present (broods 1426, 1458, 1547, 1848, 1854, and 2072), with the exception of brood

TABLE 2

Variations in leighi-cenea *dominance*

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Males	*cenea* females	*leighi* females	**Recognizable *leighi/cenea* heterozygotes**	*hippo-coonides* females	*tro-phonius* females
1020	*leighi** H^Lh	H^ch	5	1	4	0	2	0
1157	*leighi* H^Lh or H^LH^c	H^ch or hh	12	1	4	0	0	0
1176	*leighi*	H^ch or hh	17	4	6	0	5	0
1192	*leighi* H^Lh	male known to be carrying *cenea*	7	4	1	0	2	0
1365	*leighi* known not to be carrying *cenea*	H^ch (presumed)	10	4	1	0	3	0
1372	***cenea***	H^Lh	**0**	**2**	**1**	**1**	**0**	**0**
1466	***leighi/cenea* heterozygote H^cH^L 1372**	**H^ch (presumed)**	**8**	**3**	**1**	**1**	**0**	**0**
1634	***leighi/cenea* heterozygote H^cH^L 1466**	**1466 H^cH^L or H^Lh or H^ch**	**0**	**0**	**0**	**2**	**0**	**0**
1740	***leighi/cenea* heterozygote H^cH^L 1634**	***hh***	**3**	**0**	**1 abnormal**	**0**	**0**	**0**
2235	*cenea* wild	male from *salaami* family†	19	0	16	0	0	8

The families in which the *leighi/cenea* heterozygotes were recognizable are in heavy type.
* See text for explanation of genetic symbols.
† See text.

1719, where a female *leighi* is known to have been mated to a male carrying *trophonius*, and 2012, 2038 and 2118 where the broods were very small. In fact the data are consistent with the hypothesis that *salaami* is formed by a combination of *trophonius* and *leighi*. Broods 1426, 1458, 1719, 1848, 1854, 2005, 2012, 2038, 2072 and 2286 are particularly relevant for here a 1:1:1:1 ratio is expected on the hypothesis that *salaami* is the heterozygote of *trophonius* and *leighi* and the combined numbers for these broods fit well with this assumption (see Table 5). It might be argued that *salaami* is recessive to all other forms but the fact that it never appears in large broods except when both *trophonius* and *leighi* are present, and that *salaami* always produces *trophonius* and/or *leighi* among its

TABLE 3

Dominance relationship of f. trophonius *to f.* hippocoonides *and f.* cenea

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Males	*cenea* females	*hippocoonides* females	*trophonius* females
1591	*trophonius** H^TH^c 1458	*hh*	13	6	0	4
1680	*trophonius*	*hh*	5	0	2	2
1705	*trophonius* H^TH^c	*hh*	12	6	0	6
1792	*trophonius* H^Th	*hh*	33	0	17	9
1807	*trophonius*	*hh*	6	0	0	2
1824	*trophonius* H^Th	H^Th	47	0	6	36

* See also Table 4.

offspring (broods 1547, 2099, 2108, 2209 and 2223) makes this interpretation almost certainly wrong (see Table 4).

Natalica was shown by FORD (1936) to be dominant to *hippocoonides*. Brood 1758 confirms this finding. Brood 2120 of a *cenea* female to a male homozygous for *hippocoonides* produced *natalica* thus demonstrating the dominance of *cenea* to *natalica*. Brood 1960 shows that *natalica* is recessive to *leighi* as indicated by the 2:1:1 ratio of *leighi* to *natalica* to *hippocoonides*. Brood 2208, one of these *leighi* mated to a male homozygous for *hippocoonides*, produced *leighi* and *natalica* thereby proving the dominance of *leighi*.

Natalica seems to interact with *trophonius* in much the same way as *leighi* does, for in broods 1743, 1987 and 2379, where both *natalica* and *trophonius* are segregating, a form of *trophonius* appears with the white of the apical patch on the forewing replaced by buff (see Figures 1–7). Such forms have been found in the wild (WELLS, personal communication) but are apparently unnamed. Such a form has never appeared except in these three broods segregating for *trophonius* and *natalica*, and the ratios in the broods are consistent with a 1:1:1:1 ratio suggesting that these abnormal *trophonius* are in fact heterozygous for *natalica* and *trophonius*. Moreover, one of these abnormal *trophonius* mated to a male not carrying *trophonius* or *natalica* (brood 1967) produced both, showing that the female parent was heterozygous for both. A second such female (1930) produced one offspring which was *trophonius*. No brood in which *trophonius* and *natalica* are segregating has failed to produce these atypical *trophonius*, (see Table 6).

Thus it appears that:

1. *hippocoonides* is recessive to all the other forms.
2. *cenea* is recessive to *trophonius* and usually to *leighi*, although the heterozygote may sometimes be recognizable.

TABLE 4

Composition of f. salaami

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Males	*cenea* females	*hippocoonides* females	*trophonius* females	*leighi* females	*salaami* females
1426	*leighi* (no *trophonius* in ancestry)	wild	9	0	0	1	4	6
1458	*trophonius* 1386*	male carrying *leighi*	22	2	0	3	6	2
1547	*salaami* 1426	1426	5	0	0	3	2	1
1673	*hippocoonides* *hh*†	H^TH^L	11	0	0	4	8	0
1719	*leighi* (no *trophonius* in ancestry)	1547	3	0	0	0	1	1
1848	*leighi* 1719	male carrying *trophonius*	11	0	1	1	3	2
1854	*leighi* H^Lh	male carrying *trophonius*	47	0	14	12	7	14
2005	*leighi* H^Lh	male carrying *trophonius*	14	0	7	5	7	2
2012	*trophonius* H^Th	male carrying *leighi*	7	0	2	0	0	3
2038	*leighi* H^Lh	1824†	1	0	1	0	0	2
2072	*leighi* 1854	1824†	7	0	2	2	2	2
2099	*salaami* 1854	*hh*	3	0	0	5	6	0
2108	*salaami* 1854	H^ch or H^cH^c	27	0	0	23	9	0
2118	*leighi* 1854	1824†	1	0	0	0	0	1
2209	*salaami* 2005	*hh*	4	0	0	1	2	0
2223	*salaami* 2012	*hh*	9	0	0	4	5	0
2286	*leighi* 1960‡	male carrying *trophonius*	20	0	0	4	7	7

* See also Table 1.
† See text on allelomorphism.
‡ See also Table 6.

TABLE 5

Totals of salaami *broods showing expected 1:1:1:1 ratio on the hypothesis that f.* salaami *is the heterozygote between f.* leighi *and f.* trophonius

Brood	*leighi*	*trophonius*	*salaami*	*cenea*	*hippocoonides*
1426	4	1	6	0	0
1458	6	3	2	2	0
1719	1	0	1	0	0
1848	3	1	2	0	1
1854	7	12	14	0	14
2005	7	5	2	0	7
2012	0	0	3	0	2
2038	0	0	2	0	1
2072	2	2	2	0	2
2286	7	4	7		
Total	37	28	41	29	

TABLE 6

Dominance relationships of f. natalica

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Males	*cenea* females	*hippo-coonides* females	*tro-phonius* females	*natalica* females	*leighi* females	Atypical *trophonius*‡ females
1743	*natalica* $H^{na}h$	H^Th	8	0	4	3	4	0	2
1758	*natalica* $H^{na}H^{na}$	*hh*	6	0	0	0	12	0	0
1930	*natalica/trophonius* 1743 $H^{na}H^T$	H^ch or *hh**	0	0	0	1	0	0	0
1960	*natalica* $H^{na}h$	H^Lh	35	0	14	0	6	30	0
1967	*natalica* $H^{na}H^T$ 1743	H^ch or *hh*	3	0	0	2	1	0	0
1987	*trophonius* H^Th 1743	1758	25	0	6	8	7	0	3
2120	*cenea* H^cH^{na}	*hh*	12	4	0	0	7	0	0
2208	*leighi* H^LH^{na} 1960	*hh*	58	0	0	0	28	31	0
2379	*trophonius*† 2223	2208	10	0	3	6	4	0	2

* Not carrying either *trophonius* or *natalica*.
† See Table 4.
‡ See text.

3. *natalica* is dominant to *hippocoonides* and recessive to *cenea* and *leighi* but produces an intermediate form with *trophonius*.
4. *leighi* is dominant to *hippocoonides*, *natalica* and nearly always to *cenea*, but it forms an intermediate (*salaami*) with *trophonius*.
5. *trophonius* is dominant to *hippocoonides* and *cenea*, but forms an intermediate with *natalica* and *leighi*.

Allelomorphism

The data taken as a whole suggest that *hippocoonides*, *cenea*, *leighi*, *trophonius* and *natalica* are all allelomorphs of one another. Thus in no instance has any female mated to a male known to be homozygous for *hippocoonides* (which is recessive to all other forms) or a male mated to a *hippocoonides* female produced more than two forms among its offspring, whereas more than two forms have appeared in many other broods. Such are 1020, 1176, 1192, 1743, 1848, 1854, 1987, 2005, 2072, details of which are shown on the tables. There are also many others which can be seen in the completed data, e.g. 1328, 1514, 1747, 1819, 1826, 1831, 1928, 1945, 2047, 2188. Secondly, if we discount *salaami* and *trophonius* with a buff apical patch (which are probably heterozygotes) more than three forms are never found within one brood. Finally including *salaami* and buff tipped *trophonius* we never find more than four forms in any one brood. That is to say we sometimes find *cenea*, *trophonius*, *leighi* and *salaami* or *hippocoonides*, *trophonius*, *leighi* and *salaami*, but never both *hippocoonides* and *cenea* with *trophonius*, *leighi* and *salaami* (Tables 2 and 4). This last combination could appear within one brood if the genes are not allelomorphs, but cannot occur if they are. However, the hypothesis that the forms are controlled by allelomorphs rests on a firmer foundation than this. Brood 1705 (Table 3) which was from a *trophonius* mother known to be heterozygous for *cenea* and mated to a male which was homozygous for *hippocoonides* gave six female *cenea* and six female *trophonius*. On the hypothesis that *cenea* and *trophonius* are not allelomorphs all the six *trophonius* females should be heterozygous for *cenea* as no *hippocoonides* appeared among the six non-*trophonius* offspring. Therefore (again on the hypothesis) all the *trophonius* should also be heterozygous for *cenea*. If, however, *trophonius* and *cenea* are controlled by allelomorphs (calling that producing *hippocoonides h*, that for *cenea* H^c and that for *trophonius* H^T), the female parent would have been H^cH^T and the male parent *hh*. Consequently, the offspring would be H^ch (*cenea*) and H^Th (*trophonius*). Thus, unlike the results if the forms are controlled by independent loci, none of the *trophonius* should be carrying the gene producing *cenea*. Brood 1824, (Table 3) in which a male of this brood (carrying *trophonius*) was mated to a female heterozygous for *trophonius*, produced 36 female *trophonius* and six female *hippocoonides* thus demonstrating ($P<0.05$) that the female from 1705 was not carrying *cenea*. These broods therefore strongly support the view that *hippocoonides*, *cenea* and *trophonius* are allelomorphs.

Brood 1854, (Table 4) segregated in a 1:1:1:1 ratio indicating that the *salaami* were heterozygous for both *leighi* and *trophonius* (see above). Two of these

salaami mated to *hippocoonides* males (broods 2099, 2108, see Table 4) produced only *trophonius* and *leighi*, making it almost certain that *leighi* and *trophonius* are allelomorphs. (Brood 1673, Table 4 is also consistent with this, the male probably being a heterozygote). Were this not so we would expect some *cenea* or *hippocoonides* to appear among their progeny. The demonstration that one of these *leighi* is not carrying *trophonius* and that the *trophonius* is not carrying *leighi* would complete the proof.

Brood 1960 (Table 6) segregated in a 2:1:1 ratio for *leighi*, *natalica* and *hippocoonides*. This demonstrates that the *natalica* must be heterozygous for *hippocoonides* and the *leighi* will either be heterozygous for *natalica* or *hippocoonides* or both, if these are not controlled by multiple allelomorphs. One of these *leighi* (brood 2208, Table 6) mated to a male homozygous for *hippocoonides* produced 31 *leighi* and 28 *natalica* and no other form. Consequently the *leighi* cannot have been heterozygous for both *natalica* and *hippocoonides* and therefore these forms must be controlled by three multiple allelomorphs. Thus we see that the evidence is strongly in favor of *hippocoonides*, *natalica*, *cenea*, *leighi* and *trophonius* being controlled by allelomorphs which we can call h, H^{na}, H^{c}, H^{l} and H^{t} respectively.

DISCUSSION

There is a representative of f. *salaami* in race *dardanus dardanus* from Southwest and Central Africa. This is form *niobe*, which mimics *Bematistes tellus* Auriv. However, *niobe* cannot always be a heterozygote between *trophonius* and *leighi* (or an allied form called *planemoides*) for it is often at too high a frequency for this to be possible. Moreover, the data quoted by FORD show that *niobe* must usually be inherited as a single unit. The most likely explanation to account for the genetics of *niobe* is that the genes that we have been calling allelomorphs are really very closely linked and that a crossover between an allelomorph controlling either *leighi* or *planemoides* and one controlling *trophonius* can give, in coupling, a combination which produces *niobe*. When there is an appropriate model, as there is for *niobe*, the coupling combination is selected for, but when there is no such model, as in South Africa, there is no such selection. Moreover, in South Africa, *planemoides* is absent and so only a crossover in a heterozygote between *leighi* and *trophonius* could give the coupling phase. Such heterozygotes will be extremely rare, probably not more frequent than one in a thousand individuals, perhaps less. Furthermore, even if a crossover occurs the product is unlikely to persist for there is no model to confer an advantage on it.

The view that the apparent allelomorphism is really due to the presence of two or more very closely linked loci controlling color and pattern is consistent with what is known about other polymorphisms. The close linkage would be expected to evolve even if not initially present if certain combinations of color and pattern controlled by separate loci are advantageous, whereas other combinations are disadvantageous (FISHER 1930; SHEPPARD 1953, 1955; KIMURA 1956).

SUMMARY

1. The genetics of all the South African (race *cenea*) polymorphic female forms of *Papilio dardanus* have been investigated. They are *hippocoonides*, *cenea* and *trophonius* which are mimetic, and *natalica*, *leighi* and *salaami* which are nonmimetic.

2. The forms all show sex-controlled inheritance appearing only in the female, which, in contrast to the monomorphic, nonmimetic males, are tailless. The genes controlling the forms behave as a multiple allelomorphic series.

3. *Salaami* is the heterozygote between *leighi* and *trophonius*.

Cenea is recessive to the latter form and dominant to *hippocoonides* and *natalica*. It is also usually recessive to *leighi* but a modifier has been found which causes the dominance to be almost completely reversed so that the heterozygote in such broods is similar to *cenea* but just distinguishable from it. *Natalica* is dominant to *hippocoonides* and recessive to *leighi* and *cenea*, and forms an intermediate heterozygote (which has no varietal name) with *trophonius*.

4. It is suggested that although the forms behave like a multiple allelomorphic series they are more probably controlled by closely linked loci and that the Central African *niobe* is controlled by the genes for *trophonius* and *leighi* or *planemoides* in coupling. In South Africa, on the other hand, where *planemoides* is absent the genes for *trophonius* and *leighi* are usually in repulsion and in the heterozygote they give *salaami*.

ACKNOWLEDGMENTS

We are greatly indebted to Dr. E. B. Ford, F.R.S., and to Dr. A. J. Cain for reading the paper and for their helpful comments.

Our thanks are due to Messrs. Gowan C. Clark, C. G. C. Dickson, D. A. Swanepoel and R. W. Wells for continuing to send us supplies of South African race *cenea*.

We are very grateful to the Nuffield Foundation for its generous support without which much of the work could not have been carried out.

LITERATURE CITED

Clarke, C. A., and P. M. Sheppard, 1959a The genetics of some mimetic forms of *Papilio dardanus*, Brown, and *Papilio glaucus*, Linn. J. Genet. **56:** 236–259.

1959b The breeding of *Papilio dardanus* Brown, in England. Technique and some results. Entomologist **92:** 89–95.

1960 The evolution of mimicry in *Papilio dardanus*. Heredity (in press).

Fisher, R. A., 1930 *The Genetical Theory of Natural Selection*. Oxford University Press. London.

Ford, E. B., 1936 The genetics of *Papilio dardanus*, Brown. Trans. Roy. Ent. Soc. London **85:** 435–466.

1953 The genetics of polymorphism in the Lepidoptera. Advances in Genet. **5:** 43–87.

KIMURA, M., 1956 A model of a genetic system which leads to closer linkage by natural selection. Evolution **10:** 278–287.

SHEPPARD, P. M., 1953 Polymorphism, linkage and the blood groups. Am. Naturalist **87:** 283–294.

1955 Genetic variability and polymorphism: synthesis. Cold Spring Harbor Symposia Quant. Biol. **20:** 271–275.

COMPARISON OF THE CONCEALED VARIABILITY IN DROSOPHILA WILLISTONI WITH THAT IN DROSOPHILA PROSALTANS

COSTAS B. KRIMBAS

Department of Zoology, Columbia University, New York, New York

Received June 18, 1959

NATURAL populations of every Drosophila species which has been studied carry genetic loads, consisting of deleterious genetic variants which are mostly recessives and are usually concealed in heterozygous condition. Many chromosomes in wild populations are lethal, semilethal, or subvital in double dose, or they cause sterility of females or of males, slower development rates, visible morphological changes, or other variations. Quantitative estimates and comparisons of the magnitudes of the genetic loads have been attempted for two pairs of closely related species of the subgenus Sophophora, namely *Drosophila pseudoobscura* and *D. persimilis* (DOBZHANSKY and SPASSKY 1953), *D. willistoni* (PAVAN, CORDEIRO, N. DOBZHANSKY, T. DOBZHANSKY, MALOGOLOWKIN, SPASSKY, and WEDEL 1951) and *D. prosaltans* (DOBZHANSKY and SPASSKY 1954). Among these, *D. pseudoobscura* seems to carry a somewhat heavier genetic load than *D. persimilis*, although the mutation rates for autosomal lethals are lower in *pseudoobscura* than in *persimilis* at similar temperatures. *D. pseudoobscura* is geographically more widely distributed, more common, successful, and ecologically more versatile than is *D. persimilis*. As for the other pair, *D. willistoni* carries more lethal, semilethal, and sterility-inducing chromosomes than *D. prosaltans*. Again, *D. willistoni* is much commoner, more successful, and ecologically more versatile than is *D. prosaltans* (DOBZHANSKY and PAVAN 1950). The published data on *D. willistoni* do not, however, give reliable information concerning the frequencies of chromosomes with recessive subvital effects in the natural populations of this species. The reason is that no satisfactory method for estimation of the frequencies of subvital chromosomes were available when PAVAN *et al.* (1951) made their study of this species. A method was evolved by WALLACE and MADDEN (1953), and was used by DOBZHANSKY and SPASSKY (1953, 1954) for making estimates of these frequencies in *D. pseudoobscura*, *D. persimilis*, and *D. prosaltans*. The chief purpose of the study reported in the present article has been to obtain comparable data for *D. willistoni*.

MATERIAL AND METHODS

A total of 177 second chromosomes of *D. willistoni* have been used in this study. They were obtained from samples of the natural populations collected in the following ten localities:

(1) Cubatão, Sao Paulo, (2) Angra dos Reis, Rio de Janeiro, (3) Mafra, Santa Catarina, collected by PROF. C. PAVAN and his collaborators, (4) Palmira, Co-

lombia, (5) Llanos near Villavicencio, Colombia, (6) Buenaventura, Colombia, collected by PROF. TH. DOBZHANSKY, (7) Panama, (8) Jamaica, (9) Trinidad, collected by PROF. M. WHEELER and DR. M. WASSERMAN, of the University of Texas, and (10) Tikal, Guatemala, collected by MRS. SOPHIE DOBZHANSKY COE (shown in Table 2).

The wild flies, or their offspring (the culture being continuously intercrossed to avoid inbreeding) were kept at temperature 15°C before being used for the experiments. No culture was kept for more than four months (about four generations).

The series of crosses used in the present experiments for analysis of the genetic contents of the second chromosomes of *D. willistoni* were the same as those used by PAVAN *et al.* (1951). One or more wild males were crossed to females homozygous for the recessive marking genes abbreviated and brown (*abb bw*). A single male from each F_1 culture (heterozygous for *abb bw*) was crossed to several females which had dominant Star (*S*) and Hook (*Hk*), recessive *abb bw*, an inverted section in one second chromosome to suppress crossing over, and a balancing recessive lethal in the other second chromosome. In the next generation (F_2), six to ten females, and a small number of males, showing *S* and *Hk* were selected and inbred. These parental flies were transferred three times to obtain oviposition in fresh culture bottles. The cultures were kept at 25°C. When the F_2 flies began to hatch, four counts were made at three-day intervals, in all the cultures. The wild type (non-*S*, non-*Hk*) flies appearing in these cultures carry in duplicate (are homozygous for) certain second chromosomes derived from the natural populations.

About simultaneously with the above series of crosses, another series of "control" crosses were made. For the controls, *S Hk*/++ females and males from *different* cultures but of similar geographic origin were intercrossed. The wild type flies hatching among the progenies are heterozygous for two different second chromosomes, derived from different wild progenitors. The control crosses were handled exactly like those which were used to produce homozygous wild type flies.

Control crosses—normal viability

Seventy intercrosses producing heterozygous wild type flies were made. The results are reported in the upper line of Table 1. A total of 35,491 flies were counted in the control crosses, and 15,555, or 43.83 percent, of them were wild type. The viability of these wild type flies is normal by definition (cf. PAVAN *et al.* 1951; DOBZHANSKY and SPASSKY 1953, 1954). Since the expected Mendelian ratio of the wild type: *S Hk* flies is 33.33:66.67, it follows that the presence of the dominant marking genes *S* and *Hk* decreases the viability of the flies which carry these genes. Table 1 shows also that the mean number of flies per control culture was 221.35.

The control experiment of PAVAN *et al.* (1951) gave results slightly different from the present one, since those investigators obtained 39.91 percent of wild type flies in the cultures. This is not surprising since PAVAN *et al.* used banana-

TABLE 1

Percentages of wild type flies, average number of flies per bottle, chromosomes or combinations tested in controls, quasinormal homozygotes and lethal plus semilethal ones, as well as in all homozygotes

		Flies counted	Percent wild	Average no. of flies per bottle	Chromosomes tested	Percentage of normal viability
Controls		35,491	43.83	221.35	70	100.0
Homozygotes	quasinormal	46,764	35.19	147.98	107	80.28
	lethal	11,837	1.19	134.51	65	2.71
	Total	58,601	22.91	142.88	172	52.27

agar food in their experiments, which is probably more favorable for *D. willistoni* than is SPASSKY's cream-of-wheat—molasses medium used in the present experiments.

The average percentages of wild type flies in the four counts made in the control cultures were 47.54, 46.56, 43.71, and 40.03. These are slightly higher than the series of values obtained by PAVAN *et al.* (1951), which were 43.37, 39.01, 35.33, and 38.76 percentage.

Lethals and semilethals

As stated above, a total of 177 wild chromosomes were involved in the experiments. Of these, 172 experiments were completed successfully. The results are summarized in Table 1 and in Figure 1.

The mean percentage of wild type flies in the control (heterozygous) cultures is taken as the standard for normal viability under our experimental conditions. This percentage is, as shown in Table 1, 43.83.

In those cultures in which the wild type flies were homozygous for certain wild second chromosomes, the frequency of the wild type class varied from zero to 48

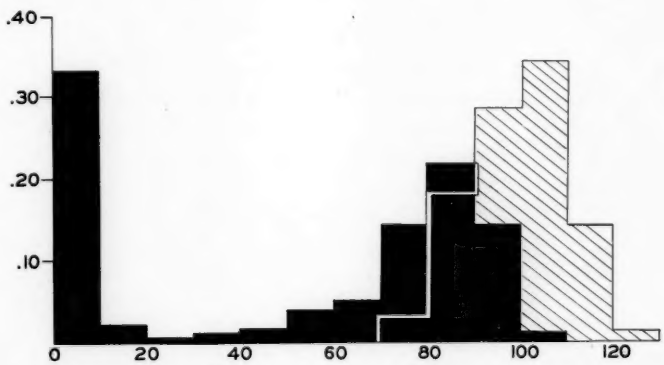


FIGURE 1.—Distribution of viability frequencies of the wild type class in homozygotes cultures (black) and in heterozygotes (crosshatched).

percent (i.e., from zero to 110 percent of normal viability). As can be seen in Figure 1, the distribution of the viability frequencies is sharply bimodal. The distribution seems to be more or less continuous from zero to 13 percent (zero to 30 percent of the normal viability). No chromosomes have been found with viabilities between 30 percent and 43 percent of normal (producing between 13 and 19 percent of wild type flies in the cultures). For viabilities from 19 to 48 percent, the observed distribution is again continuous and normal. For our purposes we have defined as semilethals those chromosomes which in homozygotes cause viability of less than 30 percent of normal. This is somewhat different from the conventional definition, in which 50 percent of the normal viability is used as the dividing line between semilethal and subvital chromosomes.

Using these criteria we found that 65 out of 177 chromosomes in our material were lethal or semilethal when homozygous (Table 2). No significant heterogeneity between different geographic localities is observed. The percentage of lethal and semilethal chromosomes in our material was 36.7 ± 3.6, which is not significantly different from 41.2 ± 1.1 given by PAVAN *et al.* (1951).

Subvital, normal and supervital chromosomes

The total number of quasinormal chromosomes adequately tested in our material was 107. Although the observed distribution is continuous, one may distinguish subvital, normal and supervital chromosomes. The estimates of the relative frequencies of these three classes were made following the method devised by WALLACE and MADDEN (1953) and used by DOBZHANSKY and SPASSKY (1953, 1954) for *D. pseudoobscura*, *D. persimilis* and *D. prosaltans*, and by DAWOOD (1958) for natural populations of *D. melanogaster*.

The observed variance (σ^2_τ) of the viabilities of the +/+ class in the experimental cultures has evidently three main components. The sampling component (σ^2_s) is estimated very easily from the numbers of flies counted in the cultures. The environmental component (σ^2_e) is estimated from the results in the three

TABLE 2

Number of chromosomes tested

Locality	Lethals and semilethals	Quasinormals	Total
Palmira	13	10	23
Llanos	9	22	31
Angra dos Reis	11	33	44
Cubatão	18	30	48
Buenaventura	2	7	9
Mafra	–	2	2
Panama	3	–	3
Guatemala	–	1	1
Jamaica	3	3	6
Trinidad	6	4	10
	65	112	177

replicate cultures which were raised to test each chromosome or chromosome combination. Subtracting the sampling and the environmental variance from the total variance observed, we obtain the residual, or genetic, variance, σ^2_g. The estimates obtained for the experimental cultures in which the +/+ class was homozygous, and for the control cultures, in which this class was heterozygous, are listed in Table 3.

The observed total variance is greater for the homozygotes than for the heterozygotes, and the difference is due largely to the real or genetic variance which is more than twice as large for the homozygotes. Following WALLACE and MADDEN, the subvital chromosomes are defined as those with viabilities less than the normal one minue $2\sigma_g$ for the control distribution. Correspondingly, the supervital chromosomes exceed the normal viability by more than $2\sigma_g$ for the control distribution. Applying these definitions to our data, the frequency of subvital chromosomes among the quasinormal array turns out to be 71.9 percent, of the normal 28.0 percent, and of the supervital chromosomes 0.1 percent.

Table 4 shows a comparison of the available estimates of the frequencies of subvital chromosomes among the quasinormals in different species of Drosophila. It should be noted that the third chromosome of *D. prosaltans* is homologous to the second chromosome of *D. pseudoobscura* and *D. persimilis*, while the second chromosomes of *D. prosaltans*, *D. willistoni* and *D. melanogaster* are homologous to the sum of the third and fourth chromosomes in *D. pseudoobscura* and *D. persimilis* (DOBZHANSKY and SPASSKY 1953, 1954). Despite the experimental errors

TABLE 3

Estimates of the components of the variance of viabilities

	σ^2_T	σ^2_s	σ^2_e	σ^2_{es}	σ^2_g	σ_g
Homozygotes	32.35	6.21	6.79	13.00	19.35	4.40
Controls	19.18	5.63	5.80	11.43	7.75	2.78

TABLE 4

Percentage frequencies of subvital chromosomes in five species

Species	Chromosome	Subvital
prosaltans	third	14.5
pseudoobscura	second	93.5
		89.6
persimilis	second	84.4
prosaltans	second	33.4
willistoni	second	71.9
pseudoobscura	third + fourth	97.3
		98.5
persimilis	third + fourth	99.5
melanogaster	second	26.1–66.6

being very considerable, it is evident that *D. prosaltans* carries fewer subvital variants in its chromosomes than do the other species.

Variability and viability

As indicated above, the quasinormal homozygotes and controls were tested generally in three replicate cultures. The variation between the percentages of the wild type class in the replicate cultures is due to sampling errors and to micro-environmental variations. The question arises whether the variance due to the environmental variations (σ^2_e in Table 3) shows any relationship to the observed viabilities of the chromosomal heterozygotes and homozygotes.

The coefficients of variation, C.V. $= 100\ \sigma_e/m$, were computed for each chromosome or chromosome combination, using the mean viability, m, which is the mean percentage of wild type flies in the cultures. Coefficients of correlation were calculated between the C.V. and the m values. Similar calculations were made for the second chromosome of *D. prosaltans* using the data of DOBZHANSKY and SPASSKY (1954), kindly furnished by them for this purpose. The values obtained are shown in Table 5. It can be seen that for the homozygotes the correlation is negative, i.e., the chromosomes which lower the viability tend to show a higher variability. The correlation coefficient is however not significant for *D. prosaltans*, while it is significant for *D. willistoni*. The correlation coefficients obtained for the control experiments (heterozygotes) are not significant.

Viability effects of the chromosomes in homozygous and heterozygous condition

One of the disputed problems in population genetics at present is to what extent the genetic loads in Mendelian populations are maintained by recurrent mutation and by balanced polymorphism. If the genetic load is chiefly mutational, one would expect a positive correlation between the viabilities of the heterozygotes and those of the homozygotes for the respective chromosomes. If the genetic load is balanced, one expects no correlation or even a negative one.

Figure 2, constructed as a correlation table, shows the viabilities of the heterozygotes on the ordinates and of the homozygotes on the abscissae. (The viabilities of the homozygotes entered in this figure are the arithmetic mean of the viabilities of the two chromosomes involved). The coefficients of correlation, computed for the second chromosomes of *D. willistoni* and *D. prosaltans*, are as follows:

D. willistoni	-0.02 ± 0.12
D. prosaltans	$+0.25 \pm 0.12$

TABLE 5

Coefficients of correlation between coefficients of variation and viabilities

	Quasinormal homozygotes	Heterozygotes
D. willistoni	-0.28 ± 0.09	$+0.15 \pm 0.11$
D. prosaltans	-0.08 ± 0.08	$+0.10 \pm 0.11$

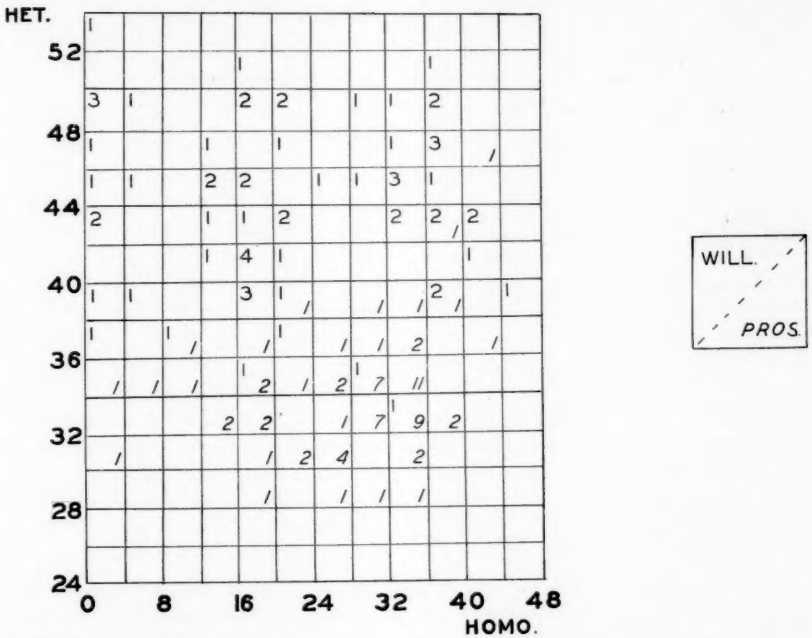


FIGURE 2.—Correlation between viabilities of the wild type class in heterozygotes and corresponding homozygotes cultures. Further explanations in the text.

A significant, though small, positive correlation is observed in *D. prosaltans*, but no correlation in *D. willistoni*. Taken at face value this would mean that the genetic load is mainly mutational in *D. prosaltans* and mainly balanced in *D. willistoni*.

DISCUSSION

Table 6 summarizes the available data concerning the frequencies of lethal, semilethal, subvital, and sterility-producing homologous chromosomes in several species. The data for *D. melanogaster*, which is a commensal species, have been omitted. It is easy to see that the population of *D. willistoni* from the central part of its geographic distribution, and those of *D. pseudoobscura* and *D. persimilis* carry heavier genetic loads than the marginal populations of *D. willistoni* and those of *D. prosaltans*.

MORTON, CROW and MULLER (1956) tried to distinguish between the mutational and the balanced genetic load with the aid of the following method. They assume that the unfixed genes are represented in the populations usually by only two alleles. If the genetic load is balanced, the adaptive values of the homozygotes are $1 - s$ and $1 - t$, while that of the heterozygote is unity. On these as-

TABLE 6

Percentage frequencies of lethal plus semilethal, subvital, sterility-inducing chromosomes in different species of the subgenus Sophophora

Species	Locality	Lethal and semilethal	Subvitals	Sterile females	Sterile males
*D. willistoni**	Central (Brazil)	41.2 ± 1.1	—	12.6	20.1
D. willistoni†	Central (Brazil)	36.7 ± 3.6	71.9	—	—
D. willistoni‡	Marginal (La Plata)	33.4 ± 2.6	—	—	—
D. willistoni‡	Marginal (Rio Grande do Sul)	32.2 ± 1.6	—	—	—
D. willistoni§	Marginal (Florida)	31.1 ± 4.4	—	10.2 ± 3.4	70.9 ± 5.1
D. willistoni§	Marginal (Cuba)	36.0 ± 9.5	—	small number of flies tested	
D. prosaltans‖	Brazil	32.6 ± 2.7	33.4	9.2 ± 2.2	11.0 ± 2.4
D. pseudoobscura¶	California	44.4 ± 8.2 42.9 ± 7.8	97.3—98.5	17.3 ± 5.8	21.1 ± 6.8
*D. persimilis***	California	48.0 ± 6.7	99.5	30.0 ± 6.5	22.8 ± 5.6

* PAVAN *et al.* 1951.
† KRIMBAS (present study).
‡ CORDEIRO *et al.* 1958.
§ TOWNSEND 1952.
‖ CAVALCANTI 1950; DOBZHANSKY and SPASSKY 1954.
¶ DOBZHANSKY and SPASSKY 1953, and DOBZHANSKY *et al.*, unpublished.
** DOBZHANSKY and SPASSKY 1953.

sumptions, the population at equilibrium suffers a loss of fitness approximately $st/(s+t)$, whereas if the population is made completely homozygous the loss of fitness becomes $2st/(s+t)$. The ratio of the two values is two. Since the few data for human populations lead to estimates of a ratio of 10 to 25, MORTON *et al.* infer that the genetic load is in the main mutational. This inference is not justified if the variable genes are respresented by more than two alleles per locus. Suppose that a locus is represented in a population by n alleles, with equilibrium frequencies $\overline{p}_1, \overline{p}_2, \ldots \overline{p}_i, \ldots \overline{p}_n$, where $\sum_{i=1}^{n} \overline{p}_i = 1$. Assuming that all the heterozygotes have a fitness equal to unity, and the fitnesses of the homozygotes are respectively $1 - s_1, 1 - s_2, \ldots; , 1 - s_n$, $(0 \leqslant s_i < 1)$ we will have the equilibrium condition:

$$(1) \quad \frac{\overline{p}_1^2 s_1}{\overline{p}_1} = \frac{\overline{p}_2^2 s_2}{\overline{p}_2} = \ldots = \frac{\overline{p}_i^2 s_i}{\overline{p}_i} = \frac{\overline{p}_n^2 s_n}{\overline{p}_n} = \frac{\overline{p}_i^2 s_i}{\overline{p}_i} = \sum_{i=1}^{n} \overline{p}_i^2 s_i$$

which is the genetic load in the panmictic population. On the other hand it is clear that

$$(2) \quad \frac{\overline{p}_i^2 s_i}{\overline{p}_i} = p_i s_i = \overline{p}_1 s_1 = \overline{p}_2 s_2 = \frac{n \overline{p}_i s_i}{n} = \frac{\sum_{i=1}^{n} \overline{p}_i s_i}{n}$$

From (1) and (2)

$$\sum_{i=1}^{n} \overline{p}_i^2 s_i = \frac{\sum_{i=1}^{n} \overline{p}_i s_i}{n} \quad \text{or}$$

$$n = \frac{\sum_{i=1}^{n} \bar{p}_i s_i}{\sum_{i=1}^{n} \bar{p}_i^2 s_i}$$

The ratio of these genetic loads is equal to the *number of alleles n*. If we consider more than one locus the treatment is difficult, because of the epistatic interactions. The test proposed by MORTON *et al.* gives accordingly ambiguous results.

It has been pointed out by several investigators (DOBZHANSKY and WALLACE 1953; DOBZHANSKY, PAVLOVSKY, SPASSKY and SPASSKY 1955) that the heterozygotes for "wild" chromosomes tend to be better buffered against changes in environmental conditions than the corresponding homozygotes. Indeed, our data for *D. willistoni* give a mean coefficient of variation for the survival rates of the homozygotes equal to 10.20 ± 1.09; the corresponding mean for the heterozygotes is 7.34 ± 0.87; the difference, 3.36 ± 1.40, is significant at between the 5 percent and 2 percent levels. It should be noted that, as shown above, the mean viability of a homozygote is negatively correlated with the coefficient of variation of its viability in different environments. The mean viability of the quasinormal homozygotes is lower than that of the control heterozygotes. If we compare the coefficients of variation in the homozygotes producing more than 34 percent of wild type flies in the cultures, with that for the heterozygotes, the difference is no longer significant (9.85 and 7.34 respectively, a difference of 2.51 ± 1.51). The situation in *D. prosaltans* is as follows: the mean coefficient of variation for the quasinormal homozygotes is 7.32, and for the heterozygotes 6.30, an insignificant difference of 1.02 ± 1.03.

It is interesting to compare the population structures in *D. willistoni* and in *D. prosaltans*, as revealed by our data. As shown above, the genetic load is greater in the former than in the latter species. In *D. willistoni*, the viability and the variability of the quasinormal homozygotes are negatively correlated; in *D. prosaltans* there is no correlation. In *D. willistoni*, the viability of a heterozygote shows no correlation with the viabilities of the corresponding homozygotes, but in *D. prosaltans* an apparently significant positive correlation is observed. These differences are consistent with the supposition that the genetic load in *D. willistoni* is predominantly a balanced load, and in *D. prosaltans* a mutational load. In turn, this is consistent with what is known concerning the reproductive biology of these species (DOBZHANSKY and PAVAN 1950; DOBZHANSKY and SPASSKY 1954). *D. willistoni* is, in South America, an ecological dominant, while *D. prosaltans* is a rare and specialized form. In the former species, close inbreeding is probably much less frequent than in the latter. Natural selection would, then, be expected to act differently in the two species. In *D. willistoni* the genetic contents of chromosomes are selected according to the fitness they confer on heterozygous individuals, while in *D. prosaltans* the fitness of the homozygotes is also important. Studying the allelism of autosomal lethals found in natural populations, PAVAN and KNAPP (1954) concluded that the genetic population sizes in *D. willistoni* (excepting certain island populations) may be considered

effectively infinite. Corresponding data are, unfortunately, not available for *D. prosaltans*, but the known features of its ecology suggest that the genetically effective size of its population may, on the average, be small. We can conclude that the genetic structure of the population in *D. willistoni* is closer to that postulated by the balance theory, while in *D. prosaltans* it is closer to what the classical theory of the genetic structure leads one to expect. The genetic population structure is interrelated with the reproductive biology of the respective populations.

SUMMARY

The concealed genetic variability in natural populations of *Drosophila willistoni* has been examined. The frequencies of chromosomes which are lethal, semilethal or subvital when homozygous, or which produce sterility of females or of males, are much higher in *D. willistoni* than in *D. prosaltans*. In *D. willistoni*, the viability of quasinormal homozygotes shows a negative correlation with the environmental sensitivity of these homozygotes; such a correlation is absent in *D. prosaltans*. On the other hand, the correlation between the viabilities of the heterozygotes and the corresponding homozygotes does not differ from zero in *D. willistoni*, while it is positive in *D. prosaltans*. These differences may be related to the differences in the reproductive biology of the two species, since *D. willistoni* is a common, successful, and ecologically versatile form, while *D. prosaltans* is rare and probably specialized ecologically. It may be inferred that the relative importance of the mutational component of the genetic load is greater in *D. prosaltans* than in *D. willistoni*, while that of the balanced component is greater in *D. willistoni* than in *D. prosaltans*.

ACKNOWLEDGMENT

The author wishes to thank PROF. TH. DOBZHANSKY for advice and criticism. Thanks also are due to DRS. C. PAVAN, M. WHEELER, A. B. DA CUNHA, M. WASSERMAN and MRS. SOPHIE DOBZHANSKY COE for providing the material. We would like also to thank MR. and MRS. B. SPASSKY, MRS. O. PAVLOVSKY and MRS. MICHELINE KRIMBAS for technical advice.

This work was performed during the tenure of a fellowship from International Cooperation Administration (Project No. 01–101–4006 (EPA-151)) administered by the National Academy of Sciences of the United States of America.

LITERATURE CITED

CAVALCANTI, A. G. L., 1950 Contribuçao a genetica das populaçoes naturais: analise dos gens autossomicos recessivos dos chromosomos II e III de *Drosophila prosaltans* Duda. Tese da Facul. Nac. Filosofia, Univ. Brazil, Rio Janeiro. 98 pp.

CORDEIRO, A. R., I. TOWNSEND, J. A. PETERSEN, and E. C. JAEGER, 1958 Genetics of southern marginal populations of *Drosophila willistoni*. Proc. 10th Intern. Congr. Genet. **2**: 58–59.

DAWOOD, M. M., 1958 Concealed genic variability in some populations of *Drosophila melanogaster* in Egypt. Ph.D. Thesis. University of Alexandria, Egypt.

DOBZHANSKY, TH., and C. PAVAN, 1950 Local and seasonal variations in relative frequencies of species of Drosophila in Brazil. J. Animal Ecol. **19:** 1–14.

DOBZHANSKY, TH., O. PAVLOVSKY, B. SPASSKY, and N. SPASSKY, 1955 Genetics of natural populations. XXIII. Biological role of deleterious recessives in populations of *Drosophila pseudoobscura*. Genetics **40:** 781–796.

DOBZHANSKY, TH., and B. SPASSKY, 1953 Genetics of natural populations. XXI. Concealed variability in two sympatric species of Drosophila. Genetics **38:** 471–484.

1954 Genetics of natural populations. XXII. A comparison of the concealed variability in *Drosophila prosaltans* with that in other species. Genetics **39:** 472–487.

DOBZHANSKY, TH., and B. WALLACE, 1953 The genetics of homeostasis in Drosophila. Proc. Natl. Acad. Sci. U.S. **39:** 162–171.

MORTON, N. E., J. F. CROW, and H. J. MULLER, 1956 An estimate of the mutational damage in man from data on consaguineous marriages. Proc. Natl. Acad. Sci. U.S. **42:** 855–863.

PAVAN, C., A. R. CORDEIRO, N. DOBZHANSKY, TH. DOBZHANSKY, C. MALOGOLOWKIN, B. SPASSKY, and M. WEDEL, 1951 Concealed genic variability in Brazilian populations of *Drosophila willistoni*. Genetics **36:** 13–30.

PAVAN, C., and E. N. KNAPP, 1954 The genetic population structure of Brazilian *Drosophila willistoni*. Evolution **8:** 303–313.

TOWNSEND, I., 1952 Genetics of marginal populations of *Drosophila willistoni*. Evolution **6:** 428–442.

WALLACE, B., and C. MADDEN, 1953 The frequencies of sub- and supervitals in experimental populations of *Drosophila melanogaster*. Genetics **38:** 456–470.

REACTIVATION BY MANGANOUS CHLORIDE OF SPORES INACTIVATED BY NITROGEN MUSTARD

G. MORPURGO AND G. SERMONTI

International Centre for Microbiological Chemistry, Istituto Superiore di Sanità, Rome, Italy

Received July 6, 1959

THE reactivation by visible light of conidia of *Streptomyces griseus* inactivated by ultraviolet irradiation was first observed by KELNER (1949). The phenomenon, photoreactivation, was later observed in several other organisms such as bacteriophages (DULBECCO 1950), bacteria and fungi of the genera Penicillium and Neurospora (KELNER 1952).

Reactivation of cells inactivated by ultraviolet irradiation was also obtained by means of pyruvic acid and other acids of the Krebs cycle (THOMPSON, MEFFERD and WYSS 1951; HEINMETS *et al.* 1954). Up to the end of 1957, to the present authors' knowledge, no case had come to light of the reactivation of cells inactivated by chemical mutagens. At that time it was observed, again by chance (SERMONTI and MORPURGO 1958), that manganous chloride brought about reactivation in conidia of *Penicillium chrysogenum* inactivated by nitrogen mustard (HN-2). Some of the first results concerning this effect, chemoreactivation, are described at length in this paper.

MATERIALS AND METHODS

Strains: The greater part of the work was carried out on a pyridoxine requiring heterozygous diploid strain of *Penicillium chrysogenum*, XXXIV S (SERMONTI and MORPURGO 1958). Other haploid and heterozygous diploid strains of *Penicillium chrysogenum* were used in particular experiments. Experiments were also carried out on a heterozygous diploid strain of *Saccharomyces cerevisiae* kindly provided by PROFESSOR G. MAGNI of the University of Pavia, Italy, and on a heterozygous diploid strain of *Aspergillus nidulans* kindly provided by PROFESSOR G. PONTECORVO of the University of Glasgow, Scotland.

Media: The complete medium used for all the molds was based on corn steep liquor (SERMONTI 1957), while the medium used for *S. cerevisiae* was based on yeast extract, peptone casein hydrolyzate, and vitamins. Some experiments were carried out with Czapek-Dox's minimal medium.

Nitrogen mustard treatment: Methyl-bis(β-chloroethyl)amine (HN-2) was used (6.5 mM) in an aqueous solution of sodium carbonate (3.15 percent, w./v.). Treatment of the conidia was terminated by diluting the suspensions ten times in a decontaminating solution (0.6 gm glycine, 0.7 gm $NaHCO_3$, 1 liter distilled water). Details of the procedure are described elsewhere (MORPURGO and SER-

MONTI 1958). Mutagenic treatment in some cases was performed with X-ray or ultraviolet irradiation (SERMONTI and MORPURGO 1958).

RESULTS

If conidia of *Penicillium chrysogenum* are treated with HN-2 and then plated on complete agar supplemented by manganous chloride ($MnCl_2 \cdot 4H_2O$) their survival rate is regularly higher than that of treated conidia plated on the same medium in the absence of the salt. Whether or not the salt is present, conidia which do not give rise to colonies produce twisted and overbranched promycelia, no more than 100 microns long. A similar picture is shown when conidia are inactivated by X-rays.

Reactivation by different doses of $MnCl_2$. (Figure 1): Several series of Petri dishes were plated with conidia of strain XXXIV S, treated with HN-2 so as to obtain a survival rate of about one percent on complete medium. Each series of dishes contained complete agar supplemented with a different dose of $MnCl_2 \cdot 4H_2O$, ranging from zero to 50 mM. Parallel series were plated with untreated conidia of the same strain.

The appearance of colonies derived from both treated and untreated conidia was increasingly delayed with increasing doses of $MnCl_2$; whereas in the absence of the salt colonies became visible after two days' incubation at 27°C, they appeared in the presence of $MnCl_2$ (40 mM) after four or five days, with a much reduced rate of growth. Sporulation, however, was relatively unaffected. In the case of untreated conidia, the number of colonies per dish noticeably decreased

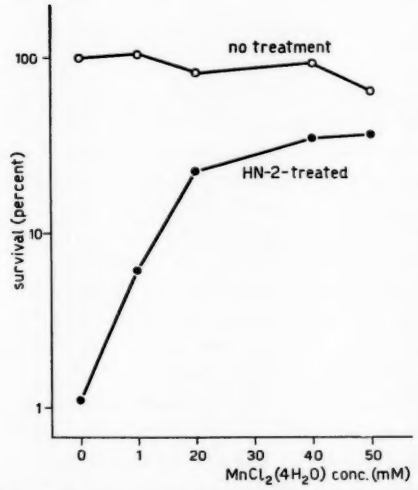


FIGURE 1.—Survival of treated (HN–2) and untreated conidia of diploid XXXIV S at different concentrations of $MnCl_2$ ($4H_2O$) in the agar.

at the higher doses of $MnCl_2$ in the agar (40–50 mM). In the case of conidia previously treated with HN-2 the number of colonies per dish increased considerably with increasing doses of $MnCl_2$. At doses higher than 50 mM growth was practically suppressed. There was a certain variation from experiment to experiment in the maxima of the survival curves due to conditions which have not yet been brought under control.

Reactivation at different HN-2-inactivation levels (Figure 2): Differences in the intensity of the HN-2 inactivation were obtained by varying the length of time, from the moment of addition of the mutagenic agent to the moment of decontamination, spent by the conidia in the HN-2 solution. The strain employed was XXXIV S. $MnCl_2$ was added to the medium at a concentration of 40 mM. The *degree of reactivation*, i.e., the number of reactivated conidia H × percent of treated conidia, decreased as the degree of inactivation increased (Table 1). In some cases where less than 95 percent of the spores were inactivated, the number of colonies obtained from platings of treated and untreated conidia on $MnCl_2$-containing medium was approximately the same (Figure 2).

Increasing the degree of HN-2-inactivation increases the *reactivation ratio*, i.e., the ratio between colonies growing from platings of the same conidial suspension in the presence and in the absence of $MnCl_2$.

Reactivation of diploid and haploid strains (Table 1, Figures 2 and 3): The first observations on the phenomenon of chemoreactivation (SERMONTI and MORPURGO 1958) suggested that there was a considerable variation in the effectiveness of reactivation with the ploidy of the strain used, the degree of reactivation being much higher with diploid strains. A number of haploid and heterozy-

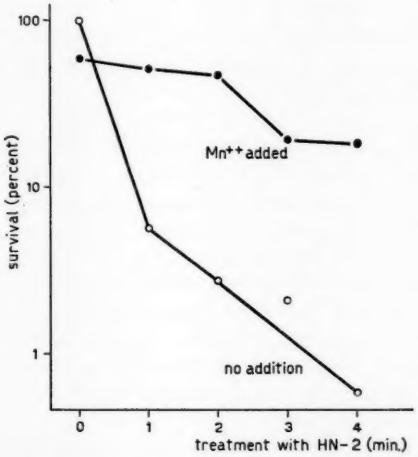


FIGURE 2.—Reactivation by Mn^{++} (40 mM) of conidia treated with nitrogen mustard (HN-2) Strain: diploid XXXIV S.

TABLE 1

Reactivation by $MnCl_2$ (40 mM) in diploid and haploid strains of Penicillium chrysogenum

Strain (code)	Ploidy	Percent survival after HN–2 treatment	Average number of colonies per dish		Reactivation ratio†	Percent reactivation‡
		without $MnCl_2$	without $MnCl_2$	with $MnCl_2$		
XXIX	diploid	0.73	31.5	363.6	11.6	7.74
		0.07	3.1	101.7	32.6	2.21
XL	diploid	0.90	90.0	1.036.0	11.5	9.45
		0.16	16.5	587.0	35.6	5.54
XXXIV S	diploid	6.00	507.0	42.5×100*	8.4	44.40
		2.77	234.3	40.3×100*	17.2	44.87
		1.61	136.0	83.3×20*	12.2	18.03
		0.58	49.0	78.2×20*	31.9	17.92
		0.46	34.0	53.8×10*	15.8	6.81
31 *py*	haploid	0.08	10.3	36.0§	3.5	0.20
		0.015	1.7	5.2§	3.0	0.03
203 *pr*	haploid	1.08	7.1	9.4	1.3	0.32
5	haploid	1.92	182.0	534.0	2.9	3.65
206 *pr*	haploid	3.01	376.6	60.2×10*	1.6	1.81
		2.09	262.0	40.7×10*	1.6	1.25
		1.18	147.3	16.4×10*	1.1	0.12
		0.77	96.2	21.8×10*	2.3	1.00

* In some experiments the $MnCl_2$ supplemented agar was plated with a more dilute conidium suspension than the unsupplemented agar. In the case of these dishes, the average is shown as the actual figure multiplied by the dilution factor.
† Average number of colonies per $MnCl_2$ supplemented dish divided by average number per unsupplemented dish.
‡ Reactivated conidia percent of treated conidia.
§ 30 mM of $MnCl_2$.

gous diploid strains (PONTECORVO and SERMONTI 1957) were treated with HN-2 and reactivated with $MnCl_2$. Diploid strains showed a much higher ratio and degree of reactivation than haploid.

Persistence of spore reactivability: Experiments were carried out to determine how long the capacity for reactivation persists in the spores after inactivation. A suspension of spores treated with HN-2 which was diluted in the usual way in the decontaminating solution, gave a survival rate on complete medium of 1.3 percent. A sample of the suspension was plated on complete medium and another on complete medium supplemented with $MnCl_2$ (40 mM), and the remainder of the suspension was kept at $+5°C$. After three weeks two more samples of the same suspension were plated on the two media. The first pair of samples produced 108 ± 8 and 755 ± 16 colonies per dish on unsupplemented and supplemented medium respectively, and the second produced 235 ± 14 and 710 ± 69. The proportion of spores capable of reactivation by $MnCl_2$ had thus not significantly altered during the storage period.

In another experiment, the HN-2 treated spore suspension was incubated in liquid nutrient medium in optimum growth conditions; samples of the suspension were taken at intervals and plated on complete agar in the presence and in

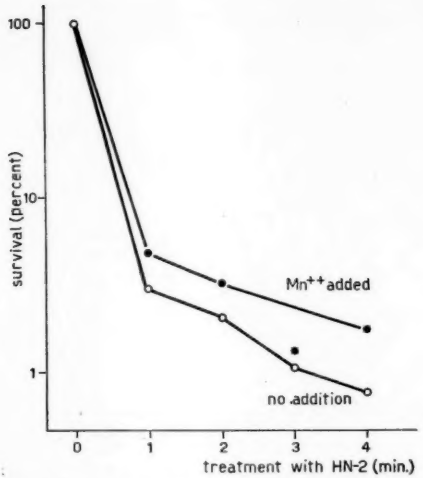


FIGURE 3.—Reactivation by Mn^{++} (40 mM) of conidia treated with nitrogen mustard (HN-2). Strain: haploid 31 *py*.

the absence of $MnCl_2$. The liquid medium contained eight percent corn steep liquor and six percent dextrin, and was used in quantities of 20 ml in 100 ml flasks shaken on a rotary shaker at 24°C. Incubation was discontinued after nine hours, since by this time germination of the conidia had started and they could form clusters and stick to the walls of the flasks. No significant change in the number of spores reactivable by $MnCl_2$ (17.4 ± 1.7 percent in five samples) was observed throughout the whole of the preincubation period. Thus three weeks of storage in distilled water at 5°C on the one hand, and nine hours of preincubation in a fermentation broth on the other, represent the maximum periods of persistence of reactivability so far established.

Duration of contact with $MnCl_2$ required for spore reactivation (Figure 4): If colonies from HN-2 treated conidia grown on $MnCl_2$ are point-transferred on to medium where the salt is absent, they resume normal growth and once more reach the normal colony size of the strain concerned (SERMONTI and MORPURGO 1958). This implies that once reactivation has taken place the presence of $MnCl_2$ is no longer required by the reactivated clones. It seemed of interest to determine how long a contact with $MnCl_2$ was required by the spores for reactivation to take place. The experiment devised was as follows.

A number of Petri dishes of eight cm diameter were filled with complete agar supplemented by $MnCl_2$ (40 mM). The agar in each dish was then covered with a cellophane film disc covering the whole surface. Some of the dishes were then plated with an HN-2 treated spore suspension, and the remainder, as a control, with an untreated suspension. Beginning immediately after plating, the cellophane disc was removed from each of a series of dishes at regular intervals of

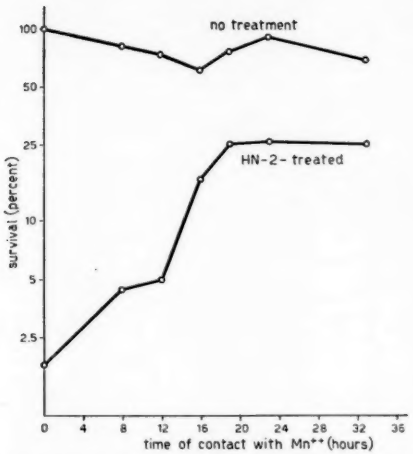


FIGURE 4.—Survival of treated (HN–2) and untreated conidia of diploid XXXIV S after different exposures to agar supplemented by $MnCl_2$ ($4H_2O$) (40 mM). The conidia are transferred from supplemented to unsupplemented agar on a disc of cellophane (see text).

time, transferred first on to Petri dishes containing complete medium and then, after at least half an hour, onto fresh dishes of the same medium. The object of this double transfer was to remove the $MnCl_2$ from the cellophane discs. Thus the conidia and microcolonies which grew on top of the cellophane where they had been in contact with $MnCl_2$, continued to grow in the absence of the salt. Results of a typical experiment are given in Figure 4. Reactivation of the treated spores increased with continued contact with $MnCl_2$ up to about the 20th hour. Further contact produced no further reactivation. Under the same conditions, no consistent effect was observed on the survival rate of untreated spores due to continued contact with $MnCl_2$ up to about 33 hours. An interesting point is that contact of the conidia with $MnCl_2$ for this period does not affect either the time of appearance or the subsequent growth of the colonies, which from this point of view are no different from colonies which have never been in contact with $MnCl_2$. The experiment described in Figure 4 was repeated three times with consistent results. The halting of the reactivation process between the 8th and the 12th hour was observed every time (Figure 4). It may be concluded that, under the experimental conditions described, the reactivation process takes about one day from seeding of the treated spores in the presence of $MnCl_2$, and that no further reactivation due to the presence of $MnCl_2$ in the medium takes place after this time.

Action of $MnCl_2$ on irradiated spores (Figure 5): The results of a typical experiment with X-ray irradiation is illustrated in Figure 5. Conidia of strain XXXIV S were given varying exposures to X-ray irradiation and plated in the presence and in the absence of $MnCl_2$. No reactivation due to the salt was ob-

served. Conidia inactivated by ultraviolet irradiation (SERMONTI and MORPURGO 1958) were also not reactivated by $MnCl_2$.

Action of other bivalent cations on HN-2 inactivated conidia (Figure 6): Some other bivalent cations were tested as Cl salts under the same conditions in which Mn^{++} produces reactivation, at different concentrations dictated by considerations of toxicity.

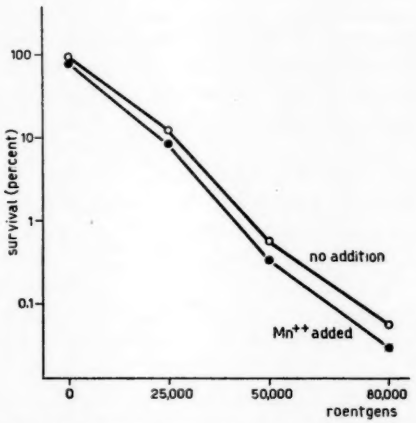


FIGURE 5.—Survival of conidia of diploid XXXIV S after different exposures to X-rays, in the presence of $MnCl_2$ ($4H_2O$) (40 mM) and in its absence. No reactivation by Mn^{++} is observed.

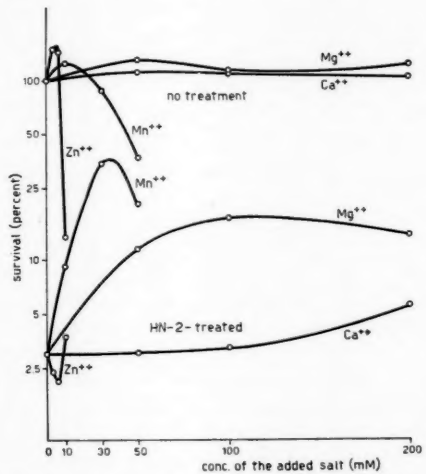


FIGURE 6.—Survival of treated (HN-2) and untreated conidia of diploid XXXIV S on agar media supplemented by different concentrations of several salts, added as chlorides.

Mg^{++}: Less toxic than Mn^{++}; produces no inactivation on untreated conidia at any of the doses used. At higher concentrations it causes a progressive decrease in the size of the colonies. It is the best reactivating agent, after Mn^{++}, of all the ions tested.

Zn^{++}: Highly toxic. The behavior of this ion is apparently paradoxical. At concentrations up to six mM it has no effect on the size of the colonies, and only slightly affects sporulation. At these dosages there is a slight increase in the number of colonies produced by treated conidia, and a slight decrease in the number of colonies produced by untreated conidia as compared in each case with the numbers produced on unsupplemented medium. At ten mM, it produces a slight reduction in the size of the colonies and further depresses sporulation, side by side with a dramatic reduction in the survival rate of the untreated conidia and an appreciable increase in that of the treated conidia.

Ca^{++}: Morphology and size of the colonies unaffected, except (slightly) at concentrations of 200 mM. The highest dose produced a slight reactivation of treated spores. A slight lowering of the survival rate of treated conidia was observed in some experiments at concentrations of Ca^{++} in the agar around 50Mm.

Reactivation by $MnCl_2$ on minimal medium: The inhibitory action of $MnCl_2$ on the growth of Penicillium is stronger on minimal than on complete medium, and for this reason it was used at lower concentrations (15 mM) in the reactivation experiments on minimal medium. Reactivation of HN-2 treated conidia of strain XXXIV S was observed on pyridoxine-supplemented minimal medium with a reactivation ratio similar to that obtained by plating the same conidial suspension on a complete medium (40 mM $MnCl_2$) also supplemented with pyridoxine ($160.4 \pm 13.1/19.8 \pm 3.1$ on minimal medium as compared with $606.6 \pm 63.6/66.2 \pm 6.0$ on complete medium. 200,000 conidia per dish). Storage overnight of HN-2 treated conidia of *P. chrysogenum* in distilled water in the presence of $MnCl_2 \cdot 4H_2O$ concentrations up to ten percent (w./v.) produced no significant increase or decrease in vitality.

$MnCl_2$ reactivation in other microorganisms (Table 2): Preliminary experiments were carried out on other genera of Eumycetes. The phenomenon of reactivation in the presence of $MnCl_2$ after inactivation by HN-2 occurred in both

TABLE 2

Reactivation by $MnCl_2$ in heterozygous diploid strains of Saccharomyces cerevisiae *and* Aspergillus nidulans

Species	$MnCl_2$ conc.	Colonies per dish (untreated) (Dilution factor)	Colonies per dish (untreated) (No.)	Colonies per dish (HN-2 treated) (No.)	Colonies per dish (HN-2 treated) (Percent survived)*	Reactivation ratio	Percent reactivation
S. cerevisiae	....	20	61.4 ± 4.9	102.2 ± 2.8	8.3	..	...
	20 mM	20	43.7 ± 12.6	262.0 ± 7.8	30.0	3.6	21.7
A. nidulans	....	50	13.7 ± 3.0	8.4 ± 0.7	1.2	..	...
	40 mM	50	16.3 ± 4.2	45.5 ± 6.0	5.6	4.7	4.4

* Percent of the untreated conidia growing on the same supplemented medium.

diploid conidia of *Aspergillus nidulans* and diploid cells of *Saccharomyces cerevisiae*.

$MnCl_2$ reactivation has been observed in *Escherichia coli* K12 and its non-occurrence in *B. megatherium*, by DR. M. PRINCIVALLE of this Institute (Personal communication).

DISCUSSION

A clear reactivating effect of $MnCl_2$ on nitrogen mustard (HN-2) inactivated conidia is established by the results presented in this paper. It would be premature to offer any explanation of the processes involved, but certain aspects call for discussion since it is possible they may provide a clue to its elucidation.

$MnCl_2$ reactivates conidia which in its absence would not produce more than an abortive promycelium, so that it must act on the conidium in the resting state or during germination. The continuous increase in the number of conidia reactivated in the first 20 hours suggests that the reactivation operates on each conidium as it sets out on a germination process which, in the absence of Mn^{++}, would abort. It is noteworthy that HN-2 treated conidia kept in a solution of $MnCl_2$ in distilled water undergo no reactivation.

It remains to be determined what type of action Mn^{++} exerts on the germination metabolism. Since germination of the conidia of *P. chrysogenum* is delayed by Mn^{++} at reactivating doses, its effect might be thought to be aspecifically inhibitory, i.e., to be the same as that of any other germination inhibitor on mutagen treated conidia. DOUDNEY and HAAS (1958) observed reactivation in ultraviolet irradiated cells of *E. coli* when the cells were incubated after irradiation in the absence of nitrogen. ALFER and GILLIES (1958) and GILLIES and ALFER (1959) also observed a reduction in the lethal effect of irradiation in *E. coli* in below optimum growth conditions or with subsequent chloramphenicol treatment. No systematic analysis has yet been carried out of the inhibiting effect on germination and the reactivating effect of different agents, but the work already reported rules out any possibility of a simple relation.

All the same, it must be considered unlikely that Mn^{++} is aspecific in its action, in view of the wide differences in the effectiveness of reactivation by Mn^{++} with different inactivating agents on the one hand and with different strains on the other. Conidia inactivated by HN-2 are the only ones which have so far been shown to be reactivable by Mn^{++}; conidia inactivated by X-ray or ultraviolet irradiation are not reactivated at all. This contrast is particularly significant in view of the close analogy between the modes of action of X-rays and HN-2, which is the most radiomimetic of all the chemical mutagens.

Mn^{++} is specific in its action from another point of view, in relation to the ploidy of the strain. Diploid strains are reactivated about ten times more effectively than haploid. No correlation was observed between ploidy and the effectiveness of photoreactivation in ultraviolet irradiated cells with haploid and diploid yeast cells (WARSHAW 1952). One may explain the effect of ploidy in Mn^{++} reactivation on the assumption that inactivation is a two-stage affair, with

a reversible process overlapped by an irreversible process, which follows immediately in haploid strains but only after further mutagen treatment in diploid strains. The haploid nucleus, certainly, is more exposed than the diploid to the lethal action of the mutagen and it may be that the irreversible inactivation takes place in the nucleus, while the reversible inactivation is sited in the extranuclear material.

ORD and DANIELLI (1956) have shown that amoeba cytoplasm is damaged lethally by an appropriate exposure to HN-2 (MBA), and that this damage is not a result of nuclear damage. They have observed that the presence of a nucleus, either during exposure or after exposure, makes no difference to the life of amoeba treated with 0.01 percent HN-2. They suggest that the nucleus is damaged in two stages; one of which is probably the direct action of HN-2, the other is presumably a result of the cytoplasmic damage.

A reduction in the frequency of chromosome rearrangements due to Mn^{++} was observed by SERMONTI and MORPURGO (1959) in *Penicillium chrysogenum* diploids after HN-2 treatment, but the reduction also occurs after X-ray irradiation, and in this case it does not accompany any increase in vitality. A restabilizing effect of Mn^{++} on unstable clones obtained after treatment of heterozygous diploids with HN-2 was also observed (SERMONTI and MORPURGO 1959). No explanation of the relation between the reactivating and the restabilizing effects of Mn^{++} can yet be offered.

No clue to the system involved in the HN-2 inactivation and Mn^{++} reactivation processes is given by the results obtained so far, except for the suggestion that a cytoplasmic site is involved. The role of Mn^{++} (or Mg^{++}) in reactivating enzymic systems inactivated after incubation of subcellular particles *in vitro* has been shown by several authors. ERNSTER (1956) demonstrated that the loss of phosphorylative capacity in isolated rat liver mitochondria can be restored by adding Mn^{++} to the inactivated system. Mg^{++} has the same effect to a smaller degree. GOSSELIN and POPJAK (1959) have shown that the enzymic activity of microsomes in sterol biosynthesis can be restored by the addition of Mn^{++} ions or by an increased concentration of Mg^{++} after inactivation by aging. In both cases the presence of ATP is required. Before attempting any interpretation of the process involved in the reactivation observed by the present authors, work at the biochemical level is badly needed.

SUMMARY

Conidia of *Penicillium chrysogenum* inactivated by nitrogen mustard (HN-2) are reactivated by the addition of $MnCl_2 \cdot 4H_2O$ to the plating agar. Reactivation is much more effective in the case of diploid strains than in that of haploid strains. The reactivability of treated conidia is unchanged after nine hours of culture in the absence of $MnCl_2$. The reactivation process is complete after about one day and further contact of the treated conidia with $MnCl_2$ does not lead to further reactivation. Conidia inactivated by X-ray or ultraviolet irradiation are not reactivated by $MnCl_2$. $MgCl_2$ also has a reactivating effect on conidia treated with

HN-2, but is less effective than $MnCl_2$. It is suggested that the site of the inactivation-reactivation process is in the cytoplasm.

ACKNOWLEDGMENTS

The authors wish to express their thanks to MR. G. GIUSEPPE for his expert technical assistance, to MR. F. G. MILES for the English translation of the Italian text. We are indebted to SIMES S.p.A., Milan, Italy, for gifts of Cloramin (HN-2).

LITERATURE CITED

ALPER, T., and N. E. GILLIES, 1958 Restoration of *Escherichia coli* strain B after irradiation: its dependence on suboptimal growth conditions. J. Gen. Microbiol. **18**: 461–472.

DOUDNEY, C. O., and F. L. HAAS, 1958 Modification of ultraviolet-induced mutation frequency and survival in bacteria by postirradiation treatment. Proc. Natl. Acad. Sci. U.S. **44**: 390–401.

DULBECCO, R., 1950 Experiments on photoreactivation of bacteriophages inactivated with ultraviolet irradiation. J. Bacteriol. **59**: 329–347.

ERNSTER, L., 1956 Organization of mitochondrial DPN-linked systems. I. Reversible uncoupling of oxidative phosphorylation. Exptl. Cell. Research **10**: 704–720.

GOSSELIN, L., and G. POPJAK, 1959 Studies on biosynthesis of cholesterol. 8. The effect of Mg^{2+} and Mn^{2+} ions and ATP on enzymic activity of microsomes in sterol biosynthesis from mevalonic acid. Biochem. J. **71**: 660–665.

GILLIES, N. E., and T. ALPER, 1959 Reduction in the lethal effect of radiations on *Escherichia coli* by treatment with chloramphenicol. Nature **183**: 237–238.

HEINMETS, F., J. J. LEHMAN, W. W. TAYLOR, and R. N. KATHAN, 1954 The study of factors which influence metabolic reactivation of the ultraviolet inactivated *Escherichia coli*. J. Bacteriol. **67**: 511–522.

KELNER, A., 1949 Effect of visible light on the recovery of *Streptomyces griseus* conidia from ultraviolet irradiation injury. Proc. Natl. Acad. Sci. U.S. **35**: 73–79.

1952 Experiments on photoreactivation with bacteria and other microorganisms. J. Cellular Comp. Physiol. **39**: (suppl. 1): 115–117.

MORPURGO, G., and G. SERMONTI, 1959 Chemically-induced instabilities in a heterozygous diploid of *Penicillium chrysogenum*. Genetics **44**: 137–152.

ORD, M. J., and J. F. DANIELLI, 1956 The site of damage in Amoebae exposed to lethal concentrations of Methyl di-(β-chloro-ethyl)-amine (a nitrogen mustard). Quart. J. Microscop. Sci. **97**: 17–28.

PONTECORVO, G., and G. SERMONTI, 1954 Parasexual recombination in *Penicillium chrysogenum*. J. Gen. Microbiol. **11**: 94–104.

SERMONTI, G., 1957 Analysis of vegetative segregation and recombination in *Penicillium chrysogenum*. Genetics **42**: 433–443.

SERMONTI, G., and G. MORPURGO, 1958 Riattivazione con cloruro di manganese di conidi inattivati con azotoiprite in *Penicillium chrysogenum*. Rend. Accad. Nazl. Lincei, Serie VIII. **26**: 96–99.

1959 Action of manganous chloride on induced somatic segregation in *Penicillium chrysogenum* diploids. Genetics **44**: 437–447.

THOMPSON, T. L., R. B. MEFFERD, and O. WYSS, 1951 The protection of bacteria by pyruvate against radiation effects. J. Bacteriol. **62**: 39–44.

WARSHAW, S. D., 1952 Effect of ploidy in photoreactivation. Proc. Soc. Exp. Biol. Med. **79**: 268–271.

INDEX

Page numbers of regular articles are given in boldface type

abnormal sex-ratio in *Drosophila willistoni*, maternally inherited, **59**
ABRAHAMSON, S., 497, 539
  oxygen influence, X-ray induction of structural changes, Drosophila oocytes, **173**
Abstracts of papers presented at the 1959 meeting of the Genetics Society of America, **497**
ADAMS, M. W., relationship of heterozygosity to homeostasis, maize, **777**
ADELBERG, EDWARD A., 497
alleles, additional, affecting red blood cell antigens, chicken, **955**
allelism among auxotrophs, Salmonella, **269**
allelism, complementation, pseudoallelism at dumpy locus, Drosophila, **347**
ALEXANDER, MARY L., 497
  biological damage from ionizing radiations, mature and immature germ cells, *Drosophila virilis*, **979**
alpha particles producing functionally stable broken chromosome ends, maize **279**
ALTENBURG, EDGAR, 498, 498
ALTENBURG, L. S., 498, 498
antigens, additional alleles, chicken, **955**
  erythrocyte, induced somatic mutations affecting, 533
ARONSON, MARGARET M., 499
ascites tumor, rat, development of cytoplasmic bodies, 511
asymmetry and variability in double bar-eyeless, **1101**
atypical pigment cell growth, hybrids of xiphophorin fishes, 499
ATZ, JAMES W., 499
average length of initial linkage blocks intact after *n* meiotic divisions within selected mating systems, 514

bacteria, induced mutation, macromolecular sequence, 506
bacterial chromosomes, organization of, 505
BADENHAUSEN, SUSAN, group of similar lethals in wild mice, **795**
BALBINDER, ELIAS, genotypic control kappa, Paramecium, **1227**
BALBINDER, EVELYN, transduction, galactose region of *Escherichia coli* K12, **309**
BAND, HENRETTA T., 499
BANGHAM, JEAN W., 532
BANIČ, S., transduction in Salmonella, **449**
barley, changes in amino acid content induced by the X-radiation of developing embryos, 526
BEARDMORE, J. A., environment affecting heterosis and homeostasis, **759**
BEATTY, A. V., 500
BEATTY, JEANNE W., 500
BECKER, G., 517
BECKER, H. J., 500
BECKSTROM, JANIS, 539
BEMIS, W. P., selective fertilization, lima beans, **555**
BENNETT, DOROTHEA, group of similar lethals in wild mice, **795**
BERGENDAHL, JANET, biological damage from ionizing radiations, mature and immature germ cells, *Drosophila virilis*, **979**
BIANCHI, A., 500
BINET, F. E., correlation due to linkage, **5**
biological damage, ionizing radiations, mature and immature germ cells, Drosophila, **979**
Blaberus discoidalis, selection for interchange heterozygosity, **251**
BLAKESLEE, ALBERT FRANCIS, biography of, **1**
BLUMEL, JOHANNA, 501
BONNIER, GERT, effects of homozygosity and heterozygosity on rate of development, *Drosophila melanogaster*, **679**
BOSE, SMRITIMOY, 501
BOWLING, MEL, 507
BRACHIONUS, male-producing lines and a maleless line in, 536
breakup of initial linkage blocks under selected mating systems, **857**
BREGNI, E., 542
BRILES, C. O., additional alleles, red blood cell antigens in chicken, **955**
BRINK, R. ALEXANDER, transpositions of modulator, a component of the variegated pericarp allele, maize, **725**
BRITTAIN, MADELEINE, biological damage from ionizing radiations, mature and immature germ cells, *Drosophila virilis*, **979**
BUCHERT, J. G., 501
BURDICK, A. B., 501
  single gene heterosis, Drosophila, **211**
BURNETT, JEAN B., 510
BURNS, SARAH N., 497
BURWELL, L. R., 517

CALVIN, M., 517
CAMPBELL, ALLAN, transduction, galactose region of *Escherichia coli* K12, **309**
CAPENOS, MARY ROWAN, 502
CARLSON, E. A., 502
  allelism, complementation, pseudoallelism at dumpy locus, Drosophila, **347**
CARSON, H. L., 503
cattle, blood groups, heritable exceptions in, 504
  immunogenetic studies of J substance in saliva, 539
Capsicum, linkage of fruit shape and color genes, **407**
cell size and number, relation to genetic and

environmental variation of body size, Drosophila, **869**
CHAPIN, M., 507
CHAO, CHUAN YING, heterotic effects of chromosomal segment, **657**
chemically induced instabilities in *Penicillium chrysogenum*, **137**
chiasma terminalization in a structural heterozygote in Secale, **705**
chicken, cytological demonstration of an X-ray induced chromosomal translocation, 529
nonadditive gene action on specific body tissues, 517
red blood cell antigens, additional alleles, **955**
red cell antigens, **14**
chromatographic analysis of free amino acids in blood, house mouse, **591**
chromosomal aberration, postirradiation modification of, 500
chromosome associations, random and non random, **1271**
chromosome pairing in salivary gland nuclei, *Drosophila melanogaster*, **163**
CHU, ERNEST H. Y., 503
CLARK, A. M., correlation due to linkage, **5**
CLARKE, C. A., genetics of Papilio, race *cenea*, **1347**
CLAYBERG, CARL D., precocious meiotic centromere division, Lycopersicon, **1335**
CLAYTON, FRANCES E., lozenge pseudoalleles effect on pigment development during pupal differentiation, *Drosophila melanogaster*, **1041**
CLIFFORD, H. T., correlation due to linkage, **5**
COCKERHAM, C. CLARK, partitions of hereditary variance, **1141**
COE, E. H., 504
COHN, NORMAN S., 527
Collinsia, effect of colchicine, 512
compound chromosomes involving X and Y, Drosophila, **187**
CONKLIN, P. M., 517
convergent improvement, corn, **341**
correlation due to linkage, **5**
CRARY, D. D., dachs gene effects upon growth of the skeleton, **609**
CRAWFORD, I. P., 504
Crepis capillaris, oxygen posttreatment and heterochromatin, X-ray induced chromosome aberrations, **124**
crossover rates, trans and cis arrangements, white locus, Drosophila, 498
CULP, THOMAS W., oil and protein content, seed coat type, Sesamum, **897**
cytogenetics of trisomic, *Matthiola incana*, **1083**

DATTA, S. P., 504
DAVIS, ROWLAND, asexual selection, Neurospora, **1291**
DE GIOVANNI, ROSALIE, 505
dehydration, effect on X-ray induced chromosomal rearrangements, Drosophila, **329**
DELAMATER, EDWARD D., 505
DEMARINIS, F., 505
asymmetry and variability in double bar-eyeless^2, **1101**
DEMEREC, M., Albert Francis Blakeslee, biography, **1**
allelism among auxotrophs, Salmonella, **269**
DESBOROUGH, SHARON, 535
diallel crosses, components of variation, **297**
DJORDJEVIC, B., 540
DNA incorporation, mammalian cells, tissue culture, 512
synthesis and the mitotic cycle, studied by autoradiography and microcinematography, 536
DOANE, W. W., 506
DOBZHANSKY, TH., genetic variability, *Drosophila prosaltans*, **75**
dominant lethality, Habrobracon and Drosophila, **1053**
DOUDNEY, C. O., 506
DOUGHERTY, ELLSWORTH C., 536
DOWNS, W. G., JR., 507
DRAY, SHELDON, 507
Drosophila, anomalous development of the tracheae in larvae, 536
anoxia delay in joining of X-ray induced breaks, 509
asymmetry and variability in double bar-eyeless, **1101**
biological damage mature and immature germ cells, ionizing radiations, **979**
body size, cell size and number in relation genetic and environmental variation of, **869**
busckii, divisibility of the nucleolus organizer and the structure of the nucleolus, 520
cellular constitution of wing, interrelations between genetic behavior and development in, **1113**
chromosome breakage and rejoining in irradiated sperm, 545
chromosome 4 effect on sex chromosome segregation, 514
chromosome instability in mitoses, w^{ve}, **923**
chromosome pairing in salivary gland nuclei, **163**
chromosomes from South Amherst population, 499
comparison of the incorporation of tritiated thymidine and cytidine into giant chromosome puffs, 531
compound chromosomes involving X and Y, **187**
concealed variability, *D. willistoni* and *D. prosaltans*, **1359**
crossover ratio, white alleles, 498
differential response to mutagens, **1149**
dominant lethality induced, **1053**
Dubinin effect and Y chromosome, **911**
effect of 2,4 dinitrophenol upon P^{32} loss in adult females, 545
effect of environment on heterosis and homeostasis, **759**
effect of low dose irradiation, 501
effect of X-rays on sperm activity, 545

effects of copper and iron salts, on viability and abnormal growths, 542
effects of deuterium oxide (heavy water), 517
effects of homozygosity and heterozygosity on rate of development, **679**
female sterility mutant, 506
genetic activity of Y chromosome, 499
genetic control of tyrosinase activity, 523
genotypic-environmental interactions, **1325**
genotypic expression conditioned by chemically defined medium, aseptic conditions, 515
germinal and somatic reversion of ivory, 522
hereditary material in spermatozoa, 502
heterosis in DDT resistant and susceptible populations, 527
homology of chromosome segments, **1027**
induced changes in female germ cells, effect of dehydration, **329**
induction of structural changes in oocytes, **173**
interaction of Hairless and engrailed with cubitus-interruptus-dominant and cubitus-interruptus-Wallace, 516
interchromosomal genotypic balance in inbred lines, 519
irradiated artificial populations, 503
lozenge pseudoalleles, eye pigment development, pupal differentiation, **1041**
maternally inherited abnormal sex-ratio, **59**
meiotic dicentrics and acentrics in inversion heterozygotes, 515
meiotic drive, cytogenetic basis of segregation distortion, **233**
melanotic masses induced by anoxia, 497
Minute rearrangements of yellow region, induction by X-rays, 511
mitochondria of Minute and wild type, 509
mutability of 18 Mev electrons, spermatozoa, **321**
mutagenic effects of quinacrine mustard, 530
mutagenicity of 2,5-bis-ethyleneimino hydroquinone, 498
mutants deficient in xanthine dehydrogenase, **153**
mutation rate, specific loci, 497
non-homologous pairing and crossing over, **1243**
non random association of inversions correlated with geographic distribution, 522
non random assortment of non-homologous chromosomes, **421**
origin of lethality in chromosome recombinants, 502
paramelanica, wild, non random associations of inversions, 538
persimilis, genetic variability, **43**
persimilis, lack of heterosis in experimental populations, 537
prosaltans, genetic variability, **75**
pseudoalleles at dumpy locus, **347**
pseudoalleles at white region, **34**
pseudoobscura, egg-laying rhythm, 545
quantitative inheritance of frequency of somatic crossing over, 543
radiation dose and dominant visible mutation rate, **967**
radiation effects on different strains, 517
reciprocal translocations between the X and Y chromosome, 529
release of genetic variability, **43**, **75**
residual effects of parental X chromosomes on white-variegation, 537
reverse mutations at forked locus, **769**
selection limits with sibmatings, **287**
sex balance in, aneuploidy of long regions of chromosome 3, 530
sex development in intersexes, 511
single gene heterosis, **211**
somatic crossing over frequency, 505
tolerance of gonial cells for heavy X-ray doses divided into installments, 527
tumorous head, genetic mechanism of maternal effect, **471**
uric acid in, 513
virilis, localized cytochemical and submicroscopic differentiations in salivary gland chromosomes, 534
willistoni, abnormal sex-ratio, **59**
xanthine dehydrogenase, maternal effect of *ma-l*$^+$ on, **547**
zeste alleles, variegation in, 500
DUBES, G. R., 507
DUNN, L. C., group of similar lethals in wild mice, **795**
DUVICK, D. N., 508

early generation analysis of lengths of heterozygous chromosome segments around a locus held heterozygous with backcrossing or selfing, **833**
effect of metabolic processes on mutation induced by alkylating agents, 540
effects of homozygosity and heterozygosity on rate of development, *Drosophila melanogaster*, **679**
EHRLICH, ELIZABETH F., 527
ELLIS, J. R., 518
EMMERLING, M. H., 508
preferential segregation in maize, **625**
ENGLESBERG, E., 508
environment affecting heterosis and homeostasis, **759**
Escherichia coli, deuterium oxide effects, 505
factors influencing postirradiation appearance of mutants, 523
gene transfer in one mating type, 544
K12, mutants of the F factor, 515
B/r, mixed gene loci controlling L-arabinose isomerase and L-ribulokinase activities, 508
protein alterations, 504
recombination and segregation in *Gal* heterogenotes showing position effect, 528
transduction of galactose region by phages, **309**
tryptophan-independent strain from tryptophan-requiring mutant, **105**
variant sex factor, 497

FABERGÉ, A. C., 509
alpha particles, production of functionally stable broken chromosome ends, maize, **279**
FAHMY, MYRTLE J., differential gene response, mutagens, Drosophila, **1149**
FAHMY, O. G., differential gene response, mutagens, Drosophila, **1149**
FALK, R., 509
FANALE, L. P., 497
FARNSWORTH, MARJORIE W., 509
fish, genetic control of pigment cell growth, 499
sex-reversal in genotype males, induced, **739**
FLING, M., 516
flower color, genic control of anthocyanins and flavonols in petals, *Impatiens balsamina*, **787**
FORSTHOEFEL, PAULINUS F., 510
FOX, ALLEN S., 510
FROST, HOWARD B., cytogenetics of a trisomic of *Matthiola incana*, involving a ring chromosome and somatic instability, **1083**
FRYE, SARA H., 511
FUCHS, MORTON S., 510
FUNG, SUI-TONG, 511

GABELMAN, W. H., 544
GAITHER, NENITA, 520
GALINSKY, I., 511
GALL, J. G., 512
GARBER, E. D., 512, 543
GARDNER, E. J., maternal effect for tumorous head, Drosophila, **471**
GARNER, ELDON J., 542
GARTLER, S. M., 512
gene conversion of cysteine mutants, Neurospora, **647**
gene, number, controlling time of maturity in *Zea mays*, **713**
genetic variability, release through recombination, **43, 75, 93**
GERSH, EILEEN SUTTON, chromosome pairing, salivary gland, Drosophila, **163**
GERSTEL, D. U., 513
GILES, NORMAN H., 503
GILMOUR, D. G., determining red cell antigens in chickens, **14**
GLASSMAN, EDWARD, maternal effect of *ma-l*$^{+}$, xanthine dehydrogenase, Drosophila, **547**
mutants deficient in xanthine dehydrogenase, **153**
GOWEN, JOHN W., 511, 513
GRAF, G. E., 513
GREEN, M. M., non-homologous pairing and crossing over in *Drosophila melanogaster*, **1243**
reverse mutations at forked locus, Drosophila, **769**
GRELL, RHODA F., 514
non random assortment of non-homologous chromosomes, Drosophila, **421**
Dubinin effect, **911**
group of similar lethals in wild mice, **795**
GRUN, P., 514
guinea pig, genetics of silvering, **387**
silvering, diminution of coat color and male sterility of white or near-white, **563**
variations in intensity of genotypes, **1001**
GUTIERREZ, MARIO G., maize, randomness of mating in isolated polycross plantings, **1075**
GUZMAN, M. A., 542

HAAS, F. L., 506
Habrobracon, dominant lethality induced, **1053**
retention of calcium^{45} in sperm, 539
HAGEN, CHARLES W., JR., flower color, genic control of anthocyanins and flavonols in petals, *Impatiens balsamina*, **787**
hamster, stable *in vitro* system having maximum proliferating ability, 546
Chinese, sex chromosomes, 519
HANSON, W. D., 514
breakup of initial linkage blocks under selected mating systems, **857**
early generation analysis of lengths of heterozygous chromosome segments around a locus held heterozygous with backcrossing or selfing, **833**
theoretical distribution of initial linkage block lengths intact in gametes of population intermated for n generations, **839**
theoretical distribution, parental gene blocks in gametes, **197**
HARRISON, E., genetics of reaction to pepper vein-banding virus, **1281**
HAVERLAND, LOREN H., 513
HAWTHORNE, D. C., 531
HeLa, karyotype and phenotype in cloned lines, **1257**
hereditary variance, partitions of, **1141**
HERSKOWITZ, I. H., mutability of 18 Mev electrons, Drosophila spermatozoa, **321**
induced changes in female germ cells, Drosophila, effect of dehydration, **329**
heterochromatin and induced chromosome aberrations in Crepis, **124**
heterosis, single gene, Drosophila, **211**
heterotic effect of chromosomal segment, **657**
HEYNE, E. G., differential sensitivity of monosomic X and the disomic to X-irradiation, **933**
differential sensitivity of 16 monosomics and disomic to single dosage, X-irradiation, **947**
HINTON, CLAUDE W., 515
chromosome instability in cleavage mitoses, Drosophila, **923**
HINTON, TAYLOR, 515
HIRAIZUMI, YUICHIRO, meiotic drive in natural populations, Drosophila, **233**
HIROTA, Y., 515
homology, terminal portion of Zea chromosome 2 and chromosome segment derived from Tripsacum, 524

HOROWITZ, N. H., 516
HOUSE, VERL L., 516
HRUBANT, H. E., 516
free amino acids in blood plasma of inbred mouse, **591**
HUGHES, A. M., 517
human, blood groups in Mayan population, 542
radiation sensitivity of chemically modified cells, 540
IKEDA, YONOSUKE, bisexual mating reaction, Saccharomyces, **375**
immobilization of antigens, Paramecium, **803**
interrelations between genetic behavior and cell constitution, Drosophila, **1113**
irradiation, interaction of localized and randomized chromatid breaks produced by X-rays and radiomimetic compounds, 527
IRWIN, M. R., 504
additional alleles, red blood cell antigens in chicken, **955**
ISHITANI, CHIYOKO, linkage data, Neurospora, **1209**
IVES, P. T., 499, 517
radiation dose and dominant visible mutation rate, Drosophila, **967**
JAAP, R. G., 517
JAMES, A. P., 518
severity of mutant effects, haploid effects in yeast, **1309**
JANICK, JULES, 518
JINKS, J. L., diallel crosses, components of variation among family means, **297**
JOHN, B., interchange heterozygosity in inbred Blaberus, **251**
JONSSON, ULLA B., effects of homozygosity and heterozygosity on rate of development, *Drosophila melanogaster*, **679**
JUDD, B. H., position pseudoalleles at white in Drosophila, **34**
KALTER, H., 518
KAPLAN, W. D., 519
KATO, REI, 519
KELLER, EDWARD C., JR., 519
KIKUDOME, GARY Y., 520
phenomenon of preferential segregation, maize, **815**
KILLEEN, N. L., 508
KIMBALL, R. F., 520
KRAWINKEL, M. E., 501
KRIMBAS, COSTAS B., comparison of concealed variability, *Drosophila willistoni* and *Drosophila prosaltans*, **1359**
KRIVSHENKO, JAKOV, 520
homology of short euchromatic elements of X and Y chromosomes of *Drosophila busckii* with the microchromosome of *Drosophila melanogaster*, **1027**

LA CHANCE, LEO E., 521, 539
LARISON, L., 535
LATTERELL, RICHARD L., 521
LAUGHLIN, JOHN S., mutability of 18 Mev. electrons, Drosophila spermatozoa, **321**
LEFEVRE, G., JR., reverse mutation studies on the forked locus in *Drosophila melanogaster*, **769**
LEONARD, MARY J., 546
LESLEY, MARGARET M., cytogenetics of a trisomic of *Matthiola incana*, involving a ring chromosome and somatic instability, **1083**
LEVENE, H., genetic variability, *Drosophila prosaltans*, **75**
genetic variability, statistical theory, **93**
LEVITAN, M., 522
LEWIS, E. B., 522
LEWIS, H. S., 523
LEWIS, H. W., 523
LEWIS, K. R., interchange heterozygosity in inbred Blaberus, **251**
LIEB, M., 523
Lilium, cytoplasmic incorporation of thymidine during meiosis and premeiosis, 541
lima beans, selective sterilization in, **555**
LINDEGREN, C. C., 535
LINDSLEY, D. L., 529
compound chromosomes, X and Y, Drosophila, **187**
LOCKE, WARREN F., cytogenetics of a trisomic of *Matthiola incana*, involving a ring chromosome and somatic instability, **1083**
lozenge, pseudoalleles, pigment development during pupal differentiation, **1041**
LUCCHESI, JOHN C., 515
Lycoris, chromosome fusion and fragmentation, 501

MA, TE-HSIU, 523
MACLEOD, H. L., 516
MACRAE, EDITH KRUGELIS, 524
MAGUIRE, MARJORIE P., 524
maize, changes in radiosensitivity of chromosomes during seed germination, 521
convergent improvement, **341**
cytoplasmic males-sterile, genic restorer of fertility, 508
functionally stable broken chromosome ends produced by alpha particles, **279**
gamma radiation, cytogenetic studies, 523
heterotic effect of chromosomal segment, **657**
heterozygosity and homeostasis relationship, **777**
Italian, new type chromosome 7, 500
modulator, component of variegated pericarp, **725**
phenomenon of preferential segregation, 520
pollen abortion, restoration of cytoplasmic male sterility, 501
preferential segregation, **815**
preferential segregation of structurally modified chromosomes, **625**
randomness of mating, **1075**
time of conversion-type event, B locus, 504
male sterility of white or near-white combination of *si* and *dm*, guinea pig, **563**
MALING, BARBARA, 525
linkage data, Neurospora, **1215**

MALOGOLOWKIN, CHANA, maternally inherited abnormal sex-ratio in Drosophila, **59**
man, chromosome aberrations, induced by X-rays, somatic cells, 503
congenital cataracts and cerebral palsy in siblings, 501
MARGOLIN, PAUL, 525
MARK, ABRAHAM M., estimation of mutation rate, **483**
MARTIN, ALBERT, JR., 526
maternal effect for tumorous head, genetic mechanism, Drosophila, **471**
mating type frequencies, Tetrahymena, genetic factors affecting, **1173**
MCDONALD, D. J., 524
MCGIBBON, W. H., additional alleles, red blood cell antigens in chicken, **955**
MEGNET, ROLAND, 526
meiosis in triploid maize-teosinte hybrids, 542
meiotic drive in natural populations, *Drosophila melanogaster*, **233**
melanotic masses induced by anoxia, Drosophila, 497
MERICLE, L. W., 526
MERICLE, RAE PHELPS, 526
MERRELL, DAVID J., 527
MERZ, TIMOTHY, 527
MEYER, HELEN U., 527
mice, cataract as affected by radiant energy, 538
dwarfs in C57, 507
MITCHELL, DONALD F., 519
MITCHELL, H. K., maternal effect on *ma-l+*, xanthine dehydrogenase, Drosophila, **547**
mutants deficient in xanthine dehydrogenase, **153**
MITCHELL, MARY B., analysis of Neurospora cross, **847**
MOHAMED, ALY H., gene, number, controlling time of maturity in *Zea mays*, **713**
monohybrid segregation, **1131**
MORPURGO, GIORGIO, chemically-induced instabilities, *Penicillium chrysogenum*, **137**
manganous chloride action on induced somatic segregation, Penicillium, **437**
spores inactivated by nitrogen mustard, reactivated by manganous chloride, **1371**
MORSE, M. L., 528
mouse, correlated responses to selection for blood-pH, 544
differences in free amino acids in the blood plasma of three inbred strains, 516
free amino acids in blood, chromatographic study, **591**
influence of uterine position and other factors on the occurrence of spontaneous cleft lip, 542
radiation response, heterosis as a factor, 513
seasonal variation in frequency of cortisone-induced cleft palate, 518
second group of similar lethals, wild populations, **795**
Springville luxoid anatomy and development, 510
variegated-type position effects in, 532
MUELLER, JO ANNE, 533
MUKAI, T., 501
single gene heterosis, Drosophila, **211**
MULLER, H. J., 527
mutability of 18 Mev electrons, Drosophila spermatozoa, **321**
MUNDKUR, BALAJI, 528
MURRAY, M. W., 517
MURTY, B. R., asynapsis in plants, random and non random chromosome associations, **1271**
mutagenicity of 2,5-bis-ethyleneimino hydroquinone, Drosophila, 498
mutagens, differential gene response in Drosophila, **1149**
mutants deficient in xanthine dehydrogenase, *Drosophila melanogaster*, **153**
mutation rate, estimation of, yeast, **483**
radiation dose and dominant visible mutation rate, Drosophila, **967**

NANNEY, D. L., 529
mating type frequencies, Tetrahymena, **1173**
Neurospora, alteration of crossing over by analogues by thymine and phenylalanine, 525
analysis of a cross, **847**
asexual selection, **1291**
comparative complementation and genetic maps of *hist-3* locus, 543
gene conversion at the *nic-2* locus in crosses between strains with normal chromosomes and a strain carrying a translocation at the locus, 532
gene conversions, **647**
linkage data, **1209, 1215, 1221**
multigenic control of tyrosinase synthesis, 516
new markers, linkage data, **1185**
relationship of gene conversion to crossing over, 538
tyrosinases separable, interconversion, 510
NEWCOMER, EARL H., 529
New Policies for **Genetics, i,** No. 4, Part 1
newt, uptake of nucleic acid by precursors of oocytes, 512
NICOLETTI, B., 529
Nicotiana, effects on progeny segregation following chronic gamma irradiation of the mutable *V* and stable *R* loci, 533
segregation in raw allopolyploids, 513
NILAN, R. A., oxygen posttreatment and heterochromatin, X-ray induced chromosome aberration frequencies, *Crepis capillaris*, **124**
nitrogen mustard, inactivated spores reactivated by manganous chloride, **1371**
non-homologous pairing and crossing over, Drosophila, **1243**
NOVITSKI, E., compound chromosomes, X and Y, Drosophila, **187**

OGUR, MAURICE, direct estimation of mutation rate, **483**
OGUR, SYLVIA, direct estimation of mutation rate, **483**

OHNO, S., 519
oil and protein content and seed coat type, sesame, **897**
OKUBO, S., 540
ORIAS, E., 529
OSTER, I. I., 530
Oxalis with floral trimorphism, 509
oxygen influence on induced structural changes in oocytes, Drosophila, **173**
oxygen posttreatment and X-ray induced chromosome aberrations, Crepis, **124**
OZEKI, H., allelism among auxotrophs, Salmonella, **269**
chromosome fragments participating, transduction in Salmonella, **457**

Papilio, race *cenea*, **1347**
Paramecium, genetic control of kappa-like particles, 533
genetics of mating type determination in syngen, **7**, 541
kappa, genotypic control, **1227**
mating type inheritance and determination, 535
properties of antigens, **803**
rate of loss of premutational damage after X-irradiation, 520
parental gene blocks in gametes of F_1, **197**
PARSONS, P. A., genotypic-environmental interactions for various temperatures, Drosophila, **1325**
Penicillium chrysogenum, instabilities in heterozygous diploids, induced chemically, **137**
manganous chloride action on induced somatic segregation, **437**
PENNEBAKER, G. B., 507
PENNY, L. H., convergent improvement, corn, **341**
PERKINS, DAVID D., linkage data, Neurospora, **1209**
linkage data, Neurospora, **1221**
new markers and linkage data, Neurospora, **1185**
PETERSON, P. A., linkage of fruit shape and color genes, Capsicum, **407**
phenomenon of preferential segregation, maize, **815**
PIPKIN, SARAH BEDICHEK, 530
pleiotropy in poliovirus, 507
Pneumococcus, sites of mutations causing impaired synthesis of capsular polysaccharide, 531
porphyria, enzyme studies, 524
POULSON, D. F., maternally inherited abnormal sex-ratio in Drosophila, **59**
precocious meiotic centromere division, Lycopersicon, **1335**
PREER, JOHN R., JR., immobilization of antigens, Paramecium, **803**
preferential segregation, maize, **625**
PRICE, SAM, chiasma terminalization in a structural heterozygote in Secale, **705**
pseudoalleles, at white region, Drosophila, **34**
lozenge, pigment development during pupal differentiation, **1041**
pseudoallelism at dumpy locus, Drosophila, **347**

rabbit, effects of *Da* on growth of skeleton, **609**
γ-globulin isoantigens, genetic control, **507**
radiation induced dominant lethality, **1053**
radiation ionizing, biological damage, germ cells of Drosophila, **979**
RAMEL, CLAES, effects of homozygosity and heterozygosity on rate of development, *Drosophila melanogaster*, **679**
RAVIN, A. W., 531
reaction to pepper vein-banding virus, **1281**
RECKEL, R. P., 533
red cell antigens in chickens, segregation of genes determining, **14**
REKEMEYER, M. L., dominant lethality in Habrobracon and Drosophila, radiation induced and genetically contrived, **1053**
relationship of heterozygosity to homeostasis, maize, **777**
replication of deoxyribonucleic acid in Chalmydomonas, 540
reverse mutations at forked locus, Drosophila, **769**
ROBERTSON, FORBES W., quantitative inheritance, cell size and number in relation to body size, Drosophila, **869**
interrelations between genetic behavior and development in cellular constitution, Drosophila, **1113**
ROMAN, H., 531
ROSENTHAL, IRA M., 524
RUDKIN, G. T., 531
RUSSELL, LIANE BRAUCH, 532
RUSSELL, W. A., convergent improvement, corn, **341**

Saccharomyces, application, to tetrad-analysis-data, of principles for establishing order of genetic factors, 535
chevalieri, bisexual mating reaction, **375**
fine structure of vesicular nuclei in frozen-dried, 528
Salmonella typhimurium, allelism among auxotrophs, **269**
chromosome fragments participating in transduction, **457**
linkage order and complementation among nonidentical alleles, leucine locus, 525
transduction to penicillin and chloramphenicol resistance, **449**
SANCHEZ, M., 542
SAND, SEAWARD A., 533
SANDLER, IRIS, meiotic drive in natural populations of Drosophila, **233**
SANDLER, L., meiotic drive in natural populations of Drosophila, **233**
SAWIN, P. B., dachs gene effects upon growth of the skeleton, **609**
SCHEINBERG, S. L., 533
Schizosaccharomyces, complementation between allelic adenylosuccinaseless mutants at the *ad*$_5$ locus, 526
SCHNELLER, MYRTLE V., 533

SCHURIN, M. F., 534
SCRIMSHAW, N. S., 542
SEARS, E. R., 534
second and third chromosomes from South Amherst population, Drosophila, 499
segregation distortion, cytogenetic basis of *Drosophila melanogaster*, **233**
selection for interchange heterozygosity, *Blaberus discoidalis*, **251**
SERMONTI, GIUSEPPE, chemically-induced instabilities, *Penicillium chrysogenum*, **137**
manganous chloride action on induced somatic segregation, Penicillium, **437**
spores inactivated by nitrogen mustard, reactivated by manganous chloride, **1371**
Sesamum indicum L., oil and protein content and seed coat type, **897**
sex factor variant, *Escherichia coli*, 497
sex vesicle during meiosis in the male of *Mus musculus* and *Rattus norvegicus*, 519
SHANK, D. B., relationship of heterozygosity to homeostasis, maize, **777**
SHEPPARD, D. E., 499
SHEPPARD, P. M., genetics of Papilio, race *cenea*, **1347**
SHULT, E. E., 535
SIEGEL, A., 535
SIEGEL, R. W., 535
SIMMONDS, N. W., genetics of reaction to pepper vein-banding virus, **1281**
SIRE, MARJORIE W., oxygen posttreatment and heterochromatin, X-ray-induced chromosome aberration frequencies *Crepis capillaris*, **124**
SISKEN, JESSE E., 536
SLATIS, H. M., 536
Solanum, evolution of isolating barriers, cytoplasmic differentiation, 514
SOLBERG, BJORN, 536
SONNEBORN, T. M., 533
SOUTHIN, J. L., 502
SPASSKY, B., genetic variability, *Drosophila prosaltans*, **75**
SPASSKY, N., genetic variability, *Drosophila prosaltans*, **75**
specific gene loci mutation rates, Drosophila, 497
SPIESS, E. B., 502, 537
genetic variability, *Drosophila persimilis*, **43**
Spinacia oleracea, identification of sex chromosome, 518
SPOFFORD, JANICE B., 537
SPRAGUE, G. F., convergent improvement, corn, **341**
maize, randomness of mating in isolated polycross plantings, **1075**
ST. LAWRENCE, PATRICIA, 532
ST. JOHN, RALPH, direct estimation of mutation rate, **483**
STADLER, D. R., 538
gene conversion of cysteine mutants, Neurospora, **647**
STADLER, JANICE, 538
STADLER, JOAN, tryptophan-independent strains from tryptophan-requiring mutant, *Escherichia coli*, **105**
STALEY, NANCY A., 539
STALKER, H. D., 538
STEFFENSEN, DALE, 539
STEINITZ-SEARS, LOTTI M., 539
STEVENS, J. M., diallel crosses, components of variation among family means, **297**
STONE, W. H., 504, 539
STRAUSS, B., 540
STRICKLAND, WALTER N., linkage data, Neurospora, **1221**
SUEOKA, N., 516, 540
SUTCLIFFE, M. C., 530
SWAMINATHAN, M. S., asynapsis in plants, random and non random chromosome associations, **1271**
SWANSON, CARL P., 527
SZYBALSKI, W., 540

TAKAHASHI, TOSHIAKI, bisexual mating reaction, Saccharomyces, **375**
TAKATS, STEPHEN T., 541
TANTAWY, A. O., selection limits with sibmatings, Drosophila, **287**
TAUB, STEPHAN R., 541
TEJADA, C., 542
TEMPLE, R. W., 517
Tetrahymena pyriformis, variety 1, serotype determination in, 529
variety 8, mechanism of mating type determination, 529
theoretical distribution of initial linkage block lengths intact in gametes of population intermated for n generations, **839**
TING, Y. C., 542
tobacco mosaic virus, mutations induced with nitrous acid, 535
Tradescantia, chromosomal aberration, postirradiation modification of, 500
enhancement of chromosome aberrations by CO_2, its dependence on the linear energy transfer of various radiations, 521
transpositions of modulator, a component of the variegated pericarp allele, maize, **725**
TRASLER, DAPHNE G., 542
Tribolium confusum, sex-linked selection, 524
tryptophan-independent *E. coli*, **105**
TSUNEWAKI, KOICHIRO, differential sensitivity of monosomic X and the disomic to X-irradiation, **933**
differential sensitivity of 16 monosomics and the disomic to single dosage X-irradiation, **947**
TURNER, J. HOWARD, 542
TUVESON, R. W., 543
TYLER, W. J., 504

VAN SCHAIK, NANCY WORNER, transpositions of modulator, a component of the variegated pericarp allele, maize, **725**
variations in cytoplasmic sterility of carrot, measured by tetrad segregation in partially fertile plants, 544

VEATCH, CATHERINE C., linkage data, Neurospora, **1221**
VETUKHIV, M. O., environment affecting heterosis and homeostasis, **759**
Vibrio tyrogenus, varieties I, II, and III, 526
virulence of biochemical mutants of *Fusarium oxysporum f. pisi*, 543
VOGT, MARGUERITE, relationship between karyotype and phenotype in cloned lines, HeLa, **1257**
VON BORSTEL, R. C., dominant lethality in Habrobracon and Drosophila, radiation induced and genetically contrived, **1053**

WEAVER, ELLEN C., 543
WEBBER, B. B., 543
WEBER, E., monohybrid segregation, **1131**
WEBSTER, JUDITH, dachs gene effects upon growth of the skeleton, **609**
WEINBURG, ROGER, 544
WEINMAN, ANTONIA A., 544
WEIR, J. A., 544
wheat, derivatives of a dicentric chromosome, 539
  differential sensitivity of monosomics and disomic to X-irradiation, **933**
  differential sensitivity to single dose X-irradiation, **947**
  Neatby's virescent, a chlorophyll aberration in, 534
WILSON, STELLA M., 520
WOLFE, H. G., 544
WOLFE, SHELDON, 545
WOLFSBERG, MARILYN F., 545, 545
WRIGHT, E. Y., maternally inherited abnormal sex-ratio in Drosophila, **59**
WRIGHT, SEWALL, genetics of silvering, guinea pig, **387**
  silvering and diminution of coat color, male sterility of white or near-white, **563**
  variations in intensity of genotypes of guinea pig at birth, **1001**
w^{re} chromosome instability in cleavage mitoses, Drosophila, **923**

xanthine dehydrogenase, Drosophila, **153, 547**
X-irradiation, differential sensitivity of monosomics and disomic, wheat, **933**
  differential sensitivity to single dose, wheat monosomics and disomic, **947**
X-ray induced chromosome aberrations, Crepis, **124**
X-ray induction of structural changes in oocytes, Drosophila, **173**
X-ray, mutation rate, specific loci, Drosophila, 497

Y chromosome genetic activity, Drosophila, 499
YAMAMOTO, TOKI-O, induction of functional sex reversal in genotype males of the medaka *(Oryzias latipes)*, progenies of sex reversals, **739**
YANDERS, A. F., 545
YANOFSKY, C., 504
  trytophan-independent strains from tryptophan-requiring mutant, *Escherichia coli*, **105**
yeast, dominance and over-dominance in radiation-induced mutations affecting a quantitative character, 518
  estimation of mutation rate, **483**
  reciprocal *vs.* nonreciprocal recombination in mitotically-dividing cells, 531
  severity of mutant effects, **1309**
YERGANIAN, GEORGE, 519, 546
YOUNG, GLENDOWLYN O., 507

ZAMENHOF, STEPHEN, 505
Zea mays, genes controlling time of maturity, **713**
  R^r complex, 508
zeste alleles, variegation, Drosophila, 500

GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Founded in 1916
by
GEORGE H. SHULL
with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

VOLUME 44 — 1959

GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

EDITORIAL BOARD

CLARENCE P. OLIVER, *Managing Editor*

WILSON S. STONE, *Managing Editor*

University of Texas

GEORGE W. BEADLE
California Institute of Technology

EVERETT R. DEMPSTER
University of California

DONALD F. JONES
Connecticut Agricultural Experiment Station

R. ALEXANDER BRINK
University of Wisconsin

L. C. DUNN
Columbia University

M. M. RHOADES
Indiana University

WILLIAM E. CASTLE
University of California

JOHN W. GOWEN
Iowa State College

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

JAMES F. CROW
University of Wisconsin

A. D. HERSHEY
Carnegie Institution of Washington

CURT STERN
University of California

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Wisconsin

TABLE OF CONTENTS

VOLUME 44

	PAGE
Portrait of ALBERT FRANCIS BLAKESLEE	*Frontispiece*
DEMEREC, M., ALBERT FRANCIS BLAKESLEE	1
BINET, F. E., A. M. CLARK, and H. T. CLIFFORD, Correlation due to linkage in certain wild plants	5
GILMOUR, D. G., Segregation of genes determining red cell antigens at high levels of inbreeding in chickens	14
JUDD, B. H., Studies on some position pseudoalleles at the white region in *Drosophila melanogaster*	34
SPIESS, ELIOT B., Release of genetic variability through recombination. II. *Drosophila persimilis*	43
MALOGOLOWKIN, CHANA, D. F. POULSON, and E. Y. WRIGHT, Experimental transfer of maternally inherited abnormal sex-ratio in *Drosophila willistoni*	59
DOBZHANSKY, TH., H. LEVENE, B. SPASSKY, and N. SPASSKY, Release of genetic variability through recombination. III. *Drosophila prosaltans*	75
LEVENE, HOWARD, Release of genetic variability through recombination. IV. Statistical theory	93
STADLER, JOAN, and CHARLES YANOFSKY, Studies on a series of tryptophan-independent strains derived from a tryptophan-requiring mutant of *Escherichia coli*	105
SIRE, MARJORIE W., and R. A. NILAN, The relation of oxygen posttreatment and heterochromatin to X-ray-induced chromosome aberration frequencies in *Crepis capillaris*	124
MORPURGO, GIORGIO, and GIUSEPPE SERMONTI, Chemically-induced instabilities in heterozygous diploids of *Penicillium chrysogenum*	137
GLASSMAN, EDWARD, and H. K. MITCHELL, Mutants of *Drosophila melanogaster* deficient in xanthine dehydrogenase	153
GERSH, EILEEN SUTTON, Some observations on chromosome pairing in the salivary gland nuclei of *Drosophila melanogaster*	163
ABRAHAMSON, SEYMOUR, The influence of oxygen on the X-ray induction of structural changes in Drosophila oocytes	173
LINDSLEY, D. L., and E. NOVITSKI, Compound chromosomes involving the X and Y chromosomes of *Drosophila melanogaster*	187
HANSON, W. D., The theoretical distribution of lengths of parental gene blocks in the gametes of an F_1 individual	197
MUKAI, TERUMI, and ALLAN B. BURDICK, Single gene heterosis associated with a second chromosome recessive lethal in *Drosophila melanogaster*	211
SANDLER, L., YUICHIRO HIRAIZUMI, and IRIS SANDLER, Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of segregation-distortion	233

PAGE

JOHN, B., and K. R. LEWIS, Selection for interchange heterozygosity in an inbred culture of *Blaberus discoidalis* (Serville) 251

DEMEREC, M., and H. OZEKI, Tests for allelism among auxotrophs of *Salmonella typhimurium* 269

FABERGÉ, A. C., Production by alpha particles of functionally stable broken chromosome ends in maize 279

TANTAWY, A. O., Selection limits with sibmatings in *Drosophila melanogaster* 287

JINKS, J. L., and J. M. STEVENS, The components of variation among family means in diallel crosses 297

CAMPBELL, ALLAN, and EVELYN BALBINDER, Transduction of the galactose region of *Escherichia coli* K12 by the phages λ and λ-434 hybrid 309

HERSKOWITZ, I. H., H. J. MULLER, and JOHN S. LAUGHLIN, The mutability of 18 Mev electrons applied to Drosophila spermatozoa 321

HERSKOWITZ, IRWIN H., Induced changes in female germ cells of Drosophila. VI. The effect of dehydration upon the X-ray-induced frequencies of crossover-like exchanges and of gross chromosomal rearrangements 329

SPRAGUE, G. F., W. A. RUSSELL, and L. H. PENNY, Further studies on convergent improvement in corn 341

CARLSON, ELOF AXEL, Allelism, complementation, and pseudoallelism at the dumpy locus in *Drosophila melanogaster* 347

TAKAHASHI, TOSHIAKI, and YONOSUKE IKEDA, Bisexual mating reaction in *Saccharomyces chevalieri* 375

WRIGHT, SEWALL, On the genetics of silvering in the guinea pig with special reference to interaction and linkage 387

PETERSON, P. A., Linkage of fruit shape and color genes in Capsicum 407

GRELL, R. F., Non random assortment of non-homologous chromosomes in *Drosophila melanogaster* 421

SERMONTI, G., and G. MORPURGO, Action of manganous chloride on induced somatic segregation in *Penicillium chrysogenum* diploids 437

BANIČ, S., Transduction to penicillin and chloramphenicol resistance in *Salmonella typhimurium* 449

OZEKI, H., Chromosome fragments participating in transduction in *Salmonella typhimurium* 457

GARDNER, E. J., Genetic mechanism of maternal effect for tumorous head in *Drosophila melanogaster* 471

OGUR, MAURICE, RALPH ST. JOHN, SYLVIA OGUR and ABRAHAM M. MARK, The direct estimation of mutation rate from mutant frequency under special conditions 483

New Policies for **Genetics** i

Abstracts of Papers Presented at the 1959 Meeting of the Genetics Society of America 497

PAGE

GLASSMAN, EDWARD, and H. K. MITCHELL, Maternal effect of *ma-l*$^+$ on xanthine dehydrogenase of *Drosophila melanogaster* 547

BEMIS, W. P., Selective fertilization in lima beans 555

WRIGHT, SEWALL, Silvering (*si*) and diminution (*dm*) of coat color of the guinea pig, and male sterility of the white or near-white combination of these 563

HRUBANT, H. EVERETT, A chromatographic analysis of the free amino acids in the blood plasma of three inbred strains of the house mouse, *Mus musculus* 591

SAWIN, P. B., D. D. CRARY, and JUDITH WEBSTER, Morphogenetic studies of the rabbit. XXIII. The effects of the dachs gene *Da* (chondrodystrophy) upon linear and lateral growth of the skeleton as influenced in time 609

EMMERLING, M. H., Preferential segregation of structurally modified chromosomes in maize 625

New Policies for **Genetics** i

STADLER, DAVID R., Gene conversion of cysteine mutants in Neurospora 647

CHAO, CHUAN YING, Heterotic effects of a chromosomal segment in maize 657

BONNIER, GERT, ULLA B. JONSSON, and CLAES RAMEL, Experiments on the effects of homozygosity and heterozygosity on the rate of development in *Drosophila melanogaster* 679

PRICE, SAM, Chiasma terminalization in a structural heterozygote in Secale 705

MOHAMED, ALY H., Inheritance of quantitative characters in *Zea mays*. I. Estimation of the number of genes controlling the time of maturity 713

VAN SCHAIK, NANCY WORNER, and R. ALEXANDER BRINK, Transpositions of modulator, a component of the variegated pericarp allele in maize 725

YAMAMOTO, TOKI-O, A further study on induction of functional sex reversal in genotypic males of the medaka (*Oryzias latipes*) and progenies of sex reversals 739

New Policies for **Genetics** i

VETUKHIV, M. O., and J. A. BEARDMORE, Effect of environment upon the manifestation of heterosis and homeostasis in *Drosophila pseudoobscura* 759

LEFEVRE, G., JR., and M. M. GREEN, Reverse mutation studies on the forked locus in *Drosophila melanogaster* 769

ADAMS, M. W., and D. B. SHANK, The relationship of heterozygosity to homeostasis in maize hybrids 777

HAGEN, CHARLES W., JR., Influence of genes controlling flower color on relative quantities of anthocyanins and flavonols in petals of *Impatiens balsamina* 787

PAGE

BENNETT, DOROTHEA, L. C. DUNN, and SUSAN BADENHAUSEN, A second group of similar lethals in populations of wild house mice 795

PREER, JOHN R., JR., studies on the immobilization antigens of Paramecium. IV. Properties of the different antigens 803

KIKUDOME, GARY Y., Studies on the phenomenon of preferential segregation in maize 815

HANSON, W. D., Early generation analysis of lengths of heterozygous chromosome segments around a locus held heterozygous with backcrossing or selfing 833

HANSON, W. D., Theoretical distribution of the initial linkage block lengths intact in the gametes of a population intermated for *n* generations 839

MITCHELL, MARY B., Detailed analysis of a Neurospora cross 847

HANSON, W. D., The breakup of initial linkage blocks under selected mating systems 857

New Policies for **Genetics** i

ROBERTSON, FORBES W., Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in Drosophila 869

CULP, THOMAS W., Inheritance and association of oil and protein content and seed coat type in sesame, *Sesamum indicum* L. 897

GRELL, RHODA F., The Dubinin effect and the Y chromosome 911

HINTON, CLAUDE W., A cytological study of w^{rc} chromosome instability in cleavage mitoses of *Drosophila melanogaster* 923

TSUNEWAKI, KOICHIRO, and E. G. HEYNE, Radiological study of wheat monosomics. I. Differential sensitivity of monosomic X and the disomic to X-irradiation 933

TSUNEWAKI, KOICHIRO, and E. G. HEYNE, Radiological study of wheat monosomics. II. Differential sensitivity of 16 monosomics and the disomic to a single dosage of X-irradiation 947

BRILES, C. O., W. H. MCGIBBON, and M. R. IRWIN, Additional alleles affecting red blood cell antigens in the chicken 955

IVES, P. T., The relationship between radiation dose and dominant visible mutation rate in *Drosophila melanogaster* 967

New Policies for **Genetics** i

ALEXANDER, MARY L., JANET BERGENDAHL, and MADELEINE BRITTAIN, Biological damage in mature and immature germ cells of *Drosophila virilis* with ionizing radiations 979

WRIGHT, SEWALL, A quantitative study of variations in intensity of genotypes of the guinea pig at birth 1001

KRIVSHENKO, J., New evidence for the homology of the short euchromatic elements of the X and Y chromosomes of *Drosophila busckii* with the microchromosome of *Drosophila melanogaster* 1027

PAGE

CLAYTON, FRANCES E., The effect of lozenge pseudoalleles on eye pigmentation in *Drosophila melanogaster*. III. Pigment development during pupal differentiation 1041

VON BORSTEL, R. C., and M. L. REKEMEYER, Radiation-induced and genetically contrived dominant lethality in Habrobracon and Drosophila 1053

GUTIERREZ, MARIO G., and G. F. SPRAGUE, Randomness of mating in isolated polycross plantings of maize 1075

FROST, HOWARD B., MARGARET M. LESLEY, and WARREN F. LOCKE, Cytogenetics of a trisomic of *Matthiola incana*, involving a ring chromosome and somatic instability of singleness (versus doubleness) of flowers and shape of leaves 1083

New Policies for **Genetics** i

DEMARINIS, F., The nature of asymmetry and variability in the double bar-eyeless^2 Drosophila 1101

ROBERTSON, FORBES W., Studies in quantitative inheritance. XIII. Interrelations between genetic behavior and development in the cellular constitution of the Drosophila wing 1113

WEBER, E., The genetical analysis of characters with continuous variability on a Mendelian basis. I. Monohybrid segregation 1131

COCKERHAM, C. CLARK, Partitions of hereditary variance for various genetic models 1141

FAHMY, O. G., and MYRTLE J. FAHMY, Differential gene response to mutagens in *Drosophila melanogaster* 1149

NANNEY, D. L., Genetic factors affecting mating type frequencies in variety 1 of *Tetrahymena pyriformis* 1173

PERKINS, DAVID D., New markers and multiple point linkage data in Neurospora 1185

PERKINS, DAVID D., and CHIYOKO ISHITANI, Linkage data for group III in Neurospora 1209

MALING, BARBARA, Linkage data for group IV markers in Neurospora 1215

STRICKLAND, WALTER N., DAVID D. PERKINS, and CATHERINE C. VEATCH, Linkage data for group V markers in Neurospora 1221

BALBINDER, ELIAS, The genotypic control of kappa in *Paramecium aurelia*, syngen 4, stock 51 1227

GREEN, M. M., Non-homologous pairing and crossing over in *Drosophila melanogaster* 1243

VOGT, MARGUERITE, A study of the relationship between karyotype and phenotype in cloned lines of strain HeLa 1257

New Policies for **Genetics** i

SWAMINATHAN, M. S., and B. R. MURTY, Aspects of asynapsis in plants. I. Random and non random chromosome associations 1271

SIMMONDS, N. W., and E. HARRISON, The genetics of reaction to pepper vein-banding virus 1281
DAVIS, ROWLAND, Asexual selection in *Neurospora crassa* 1291
JAMES, ALLEN P., The spectrum of severity of mutant effects. I. Haploid effects in yeast 1309
PARSONS, P. A., Genotypic-environmental interactions for various temperatures in *Drosophila melanogaster* 1325
CLAYBERG, CARL D., Cytogenetic studies of precocious meiotic centromere division in *Lycopersicon esculentum* Mill. 1335
CLARKE, C. A., and P. M. SHEPPARD, The genetics of *Papilio dardanus*, Brown. I. Race *cenea* from South Africa 1347
KRIMBAS, COSTAS B., Comparison of the concealed variability in *Drosophila willistoni* with that in *Drosophila melanogaster* 1359
MORPURGO, G., and G. SERMONTI, Reactivation by manganous chloride of spores inactivated by nitrogen mustard 1371

ACKNOWLEDGMENT

The Editors wish to express their appreciation to the Genetics Society of America for a gift which has made it possible to increase the size of the current volume so as to include manuscripts whose publication otherwise would have had to be deferred or declined.

The Editors wish to express their appreciation for Grant G7194 from the National Science Foundation for the publication of the manuscript backlog of Genetics.

CORRIGENDA

VOLUME 44

Page 457, line 9 should read:

ichia coli K-12 and phage P1), found that markers which behave as closely linked

GENETICS SOCIETY OF AMERICA

EXECUTIVE COMMITTEE

President, KARL SAX — *Vice-President*, J. W. BOYES

Past Presidents, R. A. BRINK, C. P. OLIVER

Secretary, W. L. RUSSELL
Oak Ridge National Lab.
Oak Ridge, Tenn.

Treasurer, R. D. OWEN
Calif. Institute of Tech.,
Pasadena 4, Calif.

"The Genetics Society of America is organized to provide facilities for association and conference among students of heredity and for the encouragement of close relationship between workers in genetics and those in related subjects. All persons actively interested in any field of genetics shall be eligible to active membership. Candidates for membership must be recommended by two members of the Society. Members shall be elected by the Executive Committee." —Constitution, Article I.

Any one interested in joining the Society should write to the Secretary.

The Genetics Society publishes annually the Records of the Society containing the program and abstracts of the Annual Meeting and other items of interest to members, including a membership list at periodic intervals. Total membership of the Society was 1086 in May, 1958. Copies of the Records may be obtained from the Treasurer for $1.00. The Genetics Society has a representative on the Editorial Board of GENETICS, Tracy M. Sonneborn.

SUSTAINING MEMBERS

The Genetics Society of America established in 1949 a new type of membership, Sustaining Members. The constitution provides that "All organizations interested in any field of genetics shall be eligible to sustaining membership." The dues for sustaining members are $50.00 a year.

Money contributed by Sustaining Members is not used for running expenses of the Society, but is used for special purposes as decided by the Executive Committee. At the meeting of the Executive Committee on August 25, 1957, $500.00 was allocated to GENETICS as a means to speed publication of genetical articles or to increase the number of pages per volumes.

The following organizations became sustaining members of the Society in the years indicated:

Animal Breeding Consultants
Rt. 1, Box 97B.
Livermore, California—1958

Associated Seed Growers
New Haven, Conn.—1950

Bear Hybrids Corn Co.
Decatur, Ill.—1950

Bristol Laboratories, Inc.
P. O. Box 657
Syracuse 1, New York—1957

W. Atlee Burpee Co.
Philadelphia 32, Pa.—1950

Colonial Poultry Farms, Inc.
Pleasant Hill, Missouri—1958

DeKalb Hybrid Seed Co.
DeKalb, Ill.—1949

Ferry-Morse Seed Co.
Detroit 31, Mich.—1949

Funk Brothers Seed Co.
Bloomington, Ill.—1950

Ghostley's Poultry Farm
Anoka, Minnesota—1956

Kimber Farms, Inc.
Niles, California—1953

Moews Seed Co.
Granville, Ill.—1949

Nicholas Turkey Breeding Farms
Sonoma, California—1958

Nichols Poultry Farm
Kingston, N. H.—1950

Northrup King & Co.
Minneapolis 15, Minn.—1957

Pfister Associated Growers, Inc.
Aurora, Ill.—1957

Pioneer Hi-Bred Corn Co.
Johnston, Iowa—1949

This page is donated by GENETICS to the GENETICS SOCIETY OF AMERICA. The society and the journal are separate organizations with a common bond of interest in furthering the science of heredity.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, or in any scientific field, if of such a character as to be of primary genetic interest.

The length of manuscripts will be limited to twenty printed pages, including tables and illustrations. Longer papers may be published only by special vote of the Editorial Board and, ordinarily, only if the author agrees to bear the cost of the additional pages. In case unusually extensive tabular material cannot be printed, it will be kept on file on request, provided two copies are furnished by the author.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to be applied toward the cost of reproducing illustrations and of printing expensive tables and formulae.

Authors should submit to the Editors **two** copies of manuscripts, **typewritten** with **double spacing** throughout, including **Literature Cited, tabular material,** and **footnotes.** Manuscripts should conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a "Summary." Excessive footnotes should be avoided. Footnotes to text statements can usually be included in the text parenthetically if necessary. Legends for figures and plates should be typewritten and double spaced on separate pages. Tables should be typewritten on separate pages with double spacing throughout, should be arranged to conform to journal page size $5 \times 7\frac{3}{8}$ and should be in a form suitable for the printer to set. Material for figures should be original drawings (clear photographs usually are satisfactory for line drawings) and should be of a size permitting slight reduction on the printed page. Illustrations should be mounted in final form for engraving. Photographs and drawings substantially larger than a typewritten page are likely to be damaged in the mail and should not be sent. Reviewing of the manuscript is facilitated if photographic copies of the figures are included.

The manuscript of a published paper will not be returned unless the author so requests.

Galley proofs will be sent to authors but page proofs will not be sent. Authors should leave forwarding directions whenever they are away from the address sent with the manuscript or should make other arrangements to have the galleys corrected promptly. Send corrected galley proofs to the Editors.

Reprints should be ordered directly from the Editors at the time galley proof is returned to the Editors. The author will be billed in accordance with the schedule of charges appearing on the reprint order blank. Copies of the schedule are available from the Editors.

Manuscripts and correspondence should be addressed to the Editors of GENETICS, Experimental Science Building 122, University of Texas, Austin 12, Texas.

CONTENTS OF PRECEDING ISSUES

SEPTEMBER, 1959, PART 2

New Policies for Genetics.

ROBERTSON, FORBES W., Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in Drosophila.

CULP, THOMAS W., Inheritance and association of oil and protein content and seed coat type in sesame, *Sesamum indicum* L.

GRELL, RHODA F., The Dubinin effect and the Y chromosome.

HINTON, CLAUDE W., A cytological study of w^{vc} chromosome instability in cleavage mitoses of *Drosophila melanogaster*.

TSUNEWAKI, KOICHIRO, and E. G. HEYNE, Radiological study of wheat monosomics. I. Differential sensitivity of monosomic X and the disomic to X-irradiation.

TSUNEWAKI, KOICHIRO, and E. G. HEYNE, Radiological study of wheat monosomics. II. Differential sensitivity of 16 monosomics and the disomic to a single dosage of X-irradiation.

BRILES, C. O., W. H. MCGIBBON, and M. R. IRWIN, Additional alleles affecting red blood cell antigens in the chicken.

IVES, P. T., The relationship between radiation dose and dominant visible mutation rate in *Drosophila melanogaster*.

NOVEMBER, 1959, PART 1

New Policies for Genetics.

ALEXANDER, MARY L., JANET BERGENDAHL, and MADELEINE BRITTAIN, Biological damage in mature and immature germ cells of *Drosophila virilis* with ionizing radiations.

WRIGHT, SEWALL, A quantitative study of variations in intensity of genotypes of the guinea pig at birth.

KRIVSHENKO, J., New evidence for the homology of the short euchromatic elements of the X and Y chromosomes of *Drosophila busckii* with the microchromosome of *Drosophila melanogaster*.

CLAYTON, FRANCES E., The effect of lozenge pseudoalleles on eye pigmentation in *Drosophila melanogaster*. III. Pigment development during pupal differentiation.

VON BORSTEL, R. C., and M. L. REKEMEYER, Radiation-induced and genetically contrived dominant lethality in Habrobracon and Drosophila.

GUTIERREZ, MARIO G., and G. F. SPRAGUE, Randomness of mating in isolated polycross plantings of maize.

FROST, HOWARD B., MARGARET M. LESLEY, and WARREN F. LOCKE, Cytogenetics of a trisomic of *Matthiola incana*, involving a ring chromosome and somatic instability of singleness (versus doubleness) of flowers and shape of leaves.

NOVEMBER, 1959, PART 2

New Policies for Genetics.

DEMARINIS, F., The nature of asymmetry and variability in the double bar-eyeless^2 Drosophila.

ROBERTSON, FORBES W., Studies in quantitative inheritance. XIII. Interrelations between genetic behavior and development in the cellular constitution of the Drosophila wing.

WEBER, E., The genetical analysis of characters with continuous variability on a Mendelian basis. I. Monohybrid segregation.

COCKERHAM, C. CLARK, Partitions of hereditary variance for various genetic models.

FAHMY, O. G., and MYRTLE J. FAHMY, Differential gene response to mutagens in *Drosophila melanogaster*.

NANNEY, D. L., Genetic factors affecting mating type frequencies in variety 1 of *Tetrahymena pyriformis*.

PERKINS, DAVID D., New markers and multiple point linkage data in Neurospora.

PERKINS, DAVID D., and CHIYOKO ISHITANI, Linkage data for group III markers in Neurospora.

MALING, BARBARA, Linkage data for group IV markers in Neurospora.

STRICKLAND, WALTER N., DAVID D. PERKINS, and CATHERINE C. VEATCH, Linkage data for group V markers in Neurospora.

BALBINDER, ELIAS, The genotypic control of kappa in *Paramecium aurelia*, syngen 4, stock 51.

GREEN, M. M., Non-homologous pairing and crossing over in *Drosophila melanogaster*.

VOGT, MARGUERITE, A study of the relationship between karyotype and phenotype in cloned lines of strain HeLa.

SINGLE NUMBERS $1.50 **ANNUAL SUBSCRIPTION $8.00**

obtainable from

GENETICS, INC., BUSINESS OFFICE, EXPERIMENTAL SCIENCE BLDG. 122,

UNIVERSITY OF TEXAS, AUSTIN 12, TEXAS

C30